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(54) Title: THERMOSTABLE PHYTASES IN FEED PREPARATION AND PLANT EXPRESSION**(57) Abstract**

The use of thermostable phytases in the preparation of animal feed, and the expression in plants of such phytases. For preparation of animal feed, a thermostable phytase is added before or during the agglomeration step. Preferred processes are pelleting, extrusion and expansion. A transgenic plant expressing a thermostable phytase may be used directly in animal feed preparation.

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**Thermostable phytases in
feed preparation and plant expression**

Technical Field

5 This application relates to thermostable phytases, viz. their use in processes for the production of animal feed, and their expression in plants.

Background art

10 WO 91/14782 describes transgenic tobacco and rapeseed plants expressing a phytase derived from *Aspergillus ficum* NRRL 3135. The transgenic tobacco seeds are fed to broilers.

 US 5,824,779 describes in standard fashion how to produce transgenic alfalfa expressing the same *A. ficum* phytase, and
15 the preparation of a phytase-containing concentrate which can be used per se as an animal feed supplement.

 EP 0 556 883 B1 describes a method for preparing feed pellets based on an extrusion technique. The addition of temperature sensitive agents, one example of which is phytase,
20 takes place after extrusion of the feed pellets, and the sensitive agents are loaded onto the pellets under reduced pressure.

 As acknowledged in EP 0 556 883 B1 the loss of activity of heat-sensitive substances during feed preparation processes is a
25 well-known problem. The above EP-patent proposes to solve this problem by adding these substances under reduced pressure subsequent to the extrusion process. This solution, however, requires a liquid form of the sensitive substance, as well as the installation of additional expensive process equipment.

The present invention provides an improved process for preparing animal feed, as well as improved phytase-expressing transgenic plants.

Summary of the Invention

The present invention provides a process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the agglomeration.

Also provided is a transgenic plant or part thereof which comprises a DNA-construct encoding a thermostable phytase.

The transgenic plant or part thereof, e.g. seeds or leaves, may be used in the feed preparation process of the invention, to thereby provide - in a preferred embodiment - at the same time a nutrient (feed ingredient) and the feed additive phytase.

Brief description of the Figures

In the detailed description of the invention below, reference is made to the drawings, of which

Fig. 1 is a differential scanning calorimetry (DSC) chart of consensus phytase-1 and consensus phytase-10;

Fig. 2 a DSC of consensus phytase-10-thermo-Q50T and consensus phytase-10-thermo-Q50T-K91A;

Fig. 3 a DSC of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A;

Fig. 4 a DSC of the phytase from *A. fumigatus* ATCC 13073 and of its α -mutant; and

Fig. 5 shows the design of the consensus-phytase-1 amino acid sequence;

- Fig. 6 an alignment and the basidiomycete consensus sequence of five Basidiomycete phytases;
- Fig. 7 the design of the consensus-phytase-10 amino acid sequence;
- 5 Fig. 8 an alignment for the design of consensus-phytase-11 (all Basidiomycete phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycete sequence; still further the amino acid sequence of *A. niger* T213 was used);
- 10 Fig. 9 the DNA and amino acid sequence of consensus-phytase-1-thermo(8)-Q50T-K91A;
- Fig. 10 the DNA and amino acid sequence of Consensus-phytase-10-thermo(3)-Q50T-K91A;
- Fig. 11 the DNA and amino acid sequence of *A. fumigatus* ATCC 15 13073 α -mutant; and
- Fig. 12 the DNA and amino acid sequence of Consensus-phytase-7 which comprises the following mutations as compared to Consensus-phytase-1: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, 20 Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

Detailed description of the invention

25 In the present context a "feed" or an "animal feed" means any natural or artificial diet, meal or the like intended or suitable for being eaten, taken in, digested, by an animal. Food for human beings is included in the above definition of feed.

"Animals" include all animals, be it polygastric animals 30 (ruminants); or monogastric animals such as human beings,

poultry, swine and fish. Preferred animals are the mono-gastric animals, in particular pigs and broilers.

The concept of "feed ingredients" includes the raw materials from which a feed is to be, or is, produced; or the intended, or actual, component parts of a feed. Feed ingredients
5 for non-human animals are usually, and preferably, selected from amongst the following non-exclusive list:

plant derived products

such as seeds, grains, leaves, roots, tubers, flowers,
10 pods, husks - and they may take the form of flakes, cakes, grits, flour, and the like;

animal derived products

such as fish meal, milk powder, bone extract, meat
extract, blood extract and the like;

15 additives

such as minerals, vitamins, aroma compounds, and feed
enhancing enzymes.

Phytic acid or myo-inositol 1,2,3,4,5,6-hexakis dihydrogen
phosphate (or for short myo-inositol hexakisphosphate) is the
20 primary source of inositol and the primary storage form of
phosphate in plant seeds and grains. In the seeds of legumes it
accounts for about 70% of the phosphate content. Seeds, cereal
grains and legumes are important feed ingredients.

Phytic acid, or its salts phytates - said terms being,
25 unless otherwise indicated, in the present context used
synonymously or at random - is an anti-nutritional factor. This
is partly due to its binding of nutritionally essential ions
such as calcium, trace minerals such as manganese, and also
proteins (by electrostatic interaction). And partly due to the
30 fact that the phosphorous thereof is not nutritionally available

either, since phytic acid and its salts, phytates, are often not metabolized.

This leads to a need of supplementing food and feed preparations with e.g. inorganic phosphate.

5 The non-metabolizable phytic acid phosphorous passes through the gastrointestinal tract of such animals and is excreted with the manure, resulting in an undesirable phosphate pollution of the environment resulting e.g. in eutrophication of the water environment and extensive growth of algae.

10 Phytic acid is degradable by phytases. In the present context a "phytase" is an polypeptide or enzyme which exhibits phytase activity, viz. which catalyzes the hydrolysis of phytate (myo-inositol hexakisphosphate) to (1) myo-inositol and/or (2) mono-, di-, tri-, tetra- and/or penta-phosphates thereof and (3)
15 inorganic phosphate.

The production of phytases by plants as well as by microorganisms has been reported. Amongst the microorganisms, phytase producing bacteria as well as phytase producing fungi are known.

20 There are several descriptions of phytase producing filamentous fungi belonging to the fungal phylum of Ascomycota (ascomycetes). In particular, there are several references to phytase producing ascomycetes of the *Aspergillus* genus such as *Aspergillus terreus* (Yamada et al., 1986, Agric. Biol. Chem.
25 322:1275-1282). Also, the cloning and expression of the phytase gene from *Aspergillus niger* var. *awamori* has been described (Piddington et al., 1993, Gene 133:55-62). EP 0420358 describes the cloning and expression of a phytase of *Aspergillus ficuum* (*niger*). EP 0684313 describes the cloning and expression of
30 phytases of the ascomycetes *Aspergillus niger*, *Myceliophthora thermophila*, *Aspergillus terreus*. Still further, some partial

sequences of phytases of *Aspergillus nidulans*, *Talaromyces thermophilus*, *Aspergillus fumigatus* and another strain of *Aspergillus terreus* are given.

The cloning and expression of a phytase of *Thermomyces lanuginosus* is described in WO 97/35017.

WO 98/28409 describes the cloning and expression of several basidiomycete phytases, e.g. from *Peniophora lycii*, *Agrocybe pediades*, *Paxillus involutus* and *Trametes pubescens*.

According to the Enzyme nomenclature database ExPASy (a repository of information relative to the nomenclature of enzymes primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB) describing each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided), two different types of phytases are presently known: A so-called 3-phytase (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) and a so-called 6-phytase (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26). The 3-phytase hydrolyses first the ester bond at a 3-position, whereas the 6-phytase hydrolyzes first an ester bond at the 6-position of phytic acid. Both of these types of phytases are included in the above definition of phytase.

Many assays of phytase activity are known, and any of these can be used for the purpose of the present invention. Preferred phytase assays are included in the examples.

The concept of "agglomeration" is defined as a process in which various components are mixed under the influence of heat. The resulting product is preferably an "agglomerate" or conglomerate in which the components adhere to each other while forming a product of a satisfactory physical stability. The formation of dust from such agglomerate is an indication of its

physical stability - the less dust being formed, the better. A suitable assay for dust formation from agglomerates is ASAE standard S 269-1. A satisfactory agglomerate has below 20%, preferably below 15%, more preferably below 10%, even more
5 preferably below 6% dust.

"Under the influence of heat" means that the temperature is at least 65°C, as measured on the product at the outlet of the agglomeration unit. More preferred temperatures are at least 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at
10 least 130°C.

A preferred agglomeration process is operated at an increased pressure. The pressure is typically due to a compacting of the ingredients, optionally in combination with a reduction of the cross-sectional or throughput area. Preferably,
15 by properly adjusting process parameters such as temperature and pressure, the resulting shear forces and shear velocities are of such magnitude, that the starch- and protein-containing feed ingredients become fluid.

"Increased pressure" means increased as compared to normal
20 atmospheric pressure, and the maximum pressure as measured within the agglomeration unit.

The addition of water vapour or steam is often included in agglomeration, but not as an absolute requirement.

Agglomeration includes, but is not limited to, the well-
25 known processes called extrusion, expansion (or pressure conditioning) and pelleting (or pellet pressing).

Extrusion is i.a. described at pp. 149-153 of a handbook which is available on request from the Danish Company Sprout-Matador, Glentevej 5-7, DK-6705 Esbjerg Ø or Niels Finsensvej 4,
30 DK-7100 Vejle ("Håndbog i Pilleteringsteknik 1996"). However, in the agglomeration process of the invention, the following

process steps mentioned in the above handbook are entirely optional:

- (i) pre-treating the feed ingredients in a cascade mixer;
- (ii) cutting the product leaving the nozzle-section into pieces
- 5 (iii) of a desired size;
- (iv) acclimatizing or conditioning it;
- (v) coating it;
- (vi) drying it;
- (vii) cooling it.

10 The process of expansion (pressure conditioning) is i.a. described in the same handbook at pp. 61-66. Also for expansion, the above process steps (i)-(vi), in particular steps (i) and (vi), are entirely optional steps.

This is so also for the following process steps:

- 15 (ii') comminuting the product (using e.g. a blade granulator as shown at p. 65);
- (vii) pelleting the product (using e.g. a pellet press as shown at p. 62);

The process of pelleting is i.a. described in the same
20 handbook at pp. 71-107. Also here, steps (i)-(vii) above are entirely optional steps. These steps are i.a. described in more detail at pp. 29-70 of the above handbook.

In a preferred agglomeration process of the invention, one or more of the above mentioned further process steps (i)-(vii)
25 are included.

A particularly preferred further step is step (i).

In a most preferred embodiment, the feed-ingredients are pre-heated in a first step (a) to a temperature of at least 45°C, preferably at least 50, 55, 60, 65, 70, 75, 80 °C; and
30 then heated in a second step (b) to a temperature of at least

65°C, preferably 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or even at least 130°C.

The addition of thermostable phytase takes place before or during step (a) and/or before or during step (b).

5 Water is preferably added in step (a). More preferably, heated steam is added during the mixing of the ingredients (steps (a) and/or (b)).

Process step (a) is preferably performed in a cascade mixer (see the above cited handbook p. 44).

10 A "thermostable" phytase is a phytase which has a T_m (melting temperature) as measured on purified phytase protein by Differential Scanning Calorimetry (DSC) of at least 65°C, preferably using for the DSC a constant heating rate, more preferably of 10°C/min. In preferred embodiments, the T_m is at
15 least 66, 67, 68, 69, 70, 71, 72, 73, 74 or 75°C. Preferably, the T_m is equal to or lower than 150°C, more preferably equal to or lower than 145, 140, 135, 130, 125, 120, 115 or 110°C. Accordingly, preferred intervals of T_m are: 65-150°C, 66-150°C, - (etc.) - 75-150°C; 65-145°C, 66-145°C, - (etc.) - 75-145°C;
20 65-140°C, - (etc.) - 75-140°C; - (etc.) - 65-110°C, 66-110°C, - (etc.) - 75-110°C.

Particularly preferred ranges for T_m are the following: between 65 and 110°C; between 70 and 110°C; between 70 and 100°C; between 75 and 95°C, or between 80 and 90°C.

25 In Example 3 below, the measurement of T_m by DSC is described, and the T_m 's of a number of phytases are shown.

The optimum temperatures are also indicated, since - in the alternative - a thermostable phytase can be defined as a phytase having a temperature-optimum of at least 60°C.
30 Preferably, the optimum temperature is determined on the substrate phytate at pH 5.5, or on the substrate phytic acid at

pH 5.0. Preferred units are FYT, FTU or the units of Example 3. The phytase assay of Example 3 is most preferred.

In preferred embodiments, the optimum temperature is at least 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70°C. Preferably, 5 the optimum temperature is equal to or lower than 140°C, more preferably equal to or lower than 135, 130, 125, 120, 115, 110, 105 or 100°C. Accordingly, preferred intervals of optimum temperature are: 60-140°C, 61-140°C, - (etc.) - 70-140°C; 60-135°C, 61-135°C, - (etc.) - 70-135°C; 60-130°C, - (etc.) - 70-10 130°C; - (etc.) - 60-100°C, 61-100°C, - (etc.) - 70-100°C.

Preferred phytases of the present invention exhibit a degree of similarity or homology, preferably identity, to the complete amino acid sequence of either of the phytases mentioned below under (iii) - preferably to the complete amino acid 15 sequence of Consensus-phytase-10-thermo-Q50T-K91A - of at least 48%, preferably at least 50, 52, 55, 60, 62, 65, 67, 70, 73, 75, 77, 80, 82, 85, 88, 90, 92, 95, 98 or 99%.

The degree of similarity or homology, alternatively identity, can be determined using any alignment programme known 20 in the art. A preferred alignment programme is GAP provided in the GCG version 8 program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711) (see also Needleman, S.B. and Wunsch, C.D., (1970), Journal of 25 Molecular Biology, 48, 443-453). Using GAP with the following settings for polypeptide sequence comparison: GAP weight of 3.000 and GAP lengthweight of 0.100.

A multiple sequence alignment can be made using the program PileUp (Program Manual for the Wisconsin Package, 30 Version 8, August 1994, Genetics Computer Group, 575 Science

Drive, Madison, Wisconsin, USA 53711), with a GapWeight of 3.000 and a GapLengthWeight of 0.100.

Using the program GAP, some selected phytases exhibit the following percentage similarity (identity in brackets) to the
 5 Consensus-phytase-10-thermo(3)-Q50T-K91A amino acid sequence:

A. fumigatus ATCC-13073 α -mutant	86.7% (81.8%)
Basidiomycet consensus	64.1% (49.0%)
Consensus-phytase-1	98.7% (97.9%)
10 Consensus-phytase-10	96.6% (94.4%)
Consensus-phytase-1-thermo(8)-Q50T-K91A	97.4% (95.5%)
Consensus-phytase-11	96.5% (94.2%)
Consensus-phytase-12	92.5% (89.9%)
Consensus-phytase-7	95.5% (93.4%)

15

A "purified" phytase is essentially free of other non-phytase polypeptides, e.g. at least about 20% pure, preferably at least about 40% pure, more preferably about 60% pure, even more preferably about 80% pure, most preferably about 90% pure,
 20 and even most preferably about 95% pure, as determined by SDS-PAGE.

Preferred thermostable phytases are the so-called consensus phytases of EP 98113176.6 (EP 0897985), viz.

- (i) any thermostable phytase which is obtainable by the
 25 processes described therein;
- (ii) a phytase comprising the amino acid sequence shown in Fig. 2 thereof or any variant or mutein thereof, preferred muteins being those comprising the substitutions Q50L; Q50T; Q50G; Q50T-Y51N or Q50L-Y51N.

30

Other preferred thermostable phytases are

- (iii) a thermostable phytase which comprises at least one of the following amino acid sequence (some of which are shown in Figs. 5-12 herein), preferably the following phytases: Consensus-phytase-1 (or simply Consensus phytase);
- 5 Consensus-phytase-1-thermo(3); Consensus-phytase-1-Q50T; basidiomycete-consensus (or simply Basidio); Consensus-phytase-10 (or Fcp 10); Consensus-phytase-11 (or Consensus Seq. 11); Consensus-phytase-1-thermo(8)-Q50T-K91A; Consensus-phytase-1-thermo(8)-Q50T; Consensus-phytase-1-thermo(8);
- 10 Consensus-phytase-10-thermo(3)-Q50T-K91A; Consensus-phytase-10-thermo(3)-Q50T (sometimes, "(3)" is deleted from this expression); *Aspergillus fumigatus* ATCC 13073 phytase α -mutant; *Aspergillus fumigatus* ATCC 13073 phytase α -mutant plus the mutations E59A, S126N, R329H, S364T, G404A; *Aspergillus fumigatus*
- 15 ATCC 13073 phytase α -mutant plus the mutations E59A, K68A, S126N, R329H, S364T, G404A; Consensus-phytase-7; Consensus-phytase-12.
- (iv) as well as thermostable variants and muteins of the phytases of (iv) and (v), in particular those comprising
- 20 one or more of the following substitutions: Q50L,T,G; Q50L-Y51N; Q50T-Y51N.

The term "plant" is intended to include not only whole

25 plants as such, but also plant parts or organs, such as leaves, seeds or grains, stem, root, tubers, flowers, callus, fruits etc.; tissues, cells, protoplasts etc.; as well as any combinations or sub-combinations thereof. Plant tissue cultures and plant cell lines as well as plant protoplasts are

30 specifically included herein.

The term "transgenic plant" is a plant as defined above, which has been genetically modified, as well as its progeny and propagating material thereof having retained the genetical modification. Preferably, the transgenic plant comprises at least one specific gene introduced into an ancestral plant by recombinant gene technology. The term is not confined to a single plant variety.

The invention relates to a transgenic plant which comprises a DNA-construct encoding a thermostable phytase.

10 In a preferred embodiment the transgenic plant is a plant grouping which is characterized in that it comprises a DNA-construct encoding a thermostable phytase. The members of this plant grouping may very well possess individuality, but are clearly distinguishable from other varieties by their common
15 characteristic feature of the the thermostable phytase DNA-construct.

Accordingly, the present teaching is applicable to more than one plant variety. No naturally occurring plant varieties are included amongst the plants of the invention.

20 In another preferred embodiment the invention relates to a transgenic plant variety or a variant thereof; a transgenic plant species, a transgenic plant genus, a transgenic plant family, and/or a transgenic plant order. More preferably, plant varieties as such are disclaimed.

25 Any thermostable phytase may be used in the present invention, e.g. any wild-type phytases, genetically engineered phytases, consensus phytases, phytase muteins, and/or phytase variants. Genetically engineered phytases include, but are not limited to, phytases prepared by site-directed mutagenesis, gene
30 shuffling, random mutagenesis, etc.

The nucleotide sequence encoding a wild-type thermostable phytase may be of any origin, including mammalian, plant and microbial origin and may be isolated from these sources by conventional methods. Preferably, the nucleotide sequence is
5 derived from a microorganism, such as a fungus, e.g. a yeast or a filamentous fungus, or a bacterium. The DNA sequence encoding a thermostable phytase may be isolated from the cell producing it, using various methods well known in the art (see e.g. WO 98/28409 and EP 0897985).

10 The nucleotide sequence encoding a thermostable genetically engineered or consensus phytase, including muteins and variants thereof, may be prepared in any way, e.g. as described in Example 3 hereof and in EP 0897985.

In order to accomplish expression of the thermostable
15 phytase in a plant of the invention the nucleotide sequence encoding the phytase is inserted into an expression construct containing regulatory elements or sequences capable of directing the expression of the nucleotide sequence and, if necessary or desired, to direct secretion of the gene product or targetting
20 of the gene product to the seeds of the plant.

In order for transcription to occur the nucleotide sequence encoding the thermostable phytase is operably linked to a suitable promoter capable of mediating transcription in the plant in question. The promoter may be an inducible promoter or
25 a constitutive promoter. Typically, an inducible promoter mediates transcription in a tissue-specific or growth-stage specific manner, whereas a constitutive promoter provides for sustained transcription in all cell tissues. An example of a suitable constitutive promoter useful for the present invention
30 is the cauliflower mosaic virus 35 S promoter. Transcription initiation sequences from the tumor-inducing plasmid (Ti) of

Agrobacterium such as the octopine synthase, nopaline synthase, or mannopine synthase initiator, are further examples of preferred constitutive promoters.

Examples of suitable inducible promoters include a seed-specific promoter such as the promoter expressing alpha-amylase in wheat seeds (see Stefanov et al, Acta Biologica Hungarica Vol. 42, No. 4 pp. 323-330 (1991), a promoter of the gene encoding a rice seed storage protein such as glutelin, prolamin, globulin or albumin (Wu et al., Plant and Cell Physiology Vol. 39, No. 8 pp. 885-889 (1998)), a Vicia faba promoter from the legumin B4 and the unknown seed protein gene from Vicia faba described by Conrad U. et al, Journal of Plant Physiology Vol. 152, No. 6 pp. 708-711 (1998), the storage protein napA promoter from Brassica napus, or any other seed specific promoter known in the art, eg as described in WO 91/14772.

In order to increase the expression of the thermostable phytase it is desirable that a promoter enhancer element is used. For instance, the promoter enhancer may be an intron which is placed between the promoter and the amylase gene. The intron may be one derived from a monocot or a dicot. For instance, the intron may be the first intron from the rice Waxy (Wx) gene (Li et al., Plant Science Vol. 108, No. 2, pp. 181-190 (1995)), the first intron from the maize Ubil (Ubiquitin) gene (Vain et al., Plant Cell Reports Vol. 15, No. 7 pp. 489-494 (1996)) or the first intron from the Act1 (actin) gene. As an example of a dicot intron the chsA intron (Vain et al. op cit.) is mentioned. Also, a seed specific enhancer may be used for increasing the expression of the thermostable phytase in seeds. An example of a seed specific enhancer is the one derived from the beta-phaseolin gene encoding the major seed storage protein of bean

(*Phaseolus vulgaris*) disclosed by Vandergeest and Hall, Plant Molecular Biology Vol. 32, No. 4, pp. 579-588 (1996).

Also, the expression construct preferably contains a terminator sequence to signal transcription termination of the
5 thermostable phytase gene such as the *rbcS2'* and the *nos3'* terminators.

To facilitate selection of successfully transformed plants, the expression construct should also preferably include one or more selectable markers, e.g. an antibiotic resistance
10 selection marker or a selection marker providing resistance to a herbicide. One widely used selection marker is the neomycin phosphotransferase gene (NPTII) which provides kanamycin resistance. Examples of other suitable markers include a marker providing a measurable enzyme activity, e.g. dihydrofolate
15 reductase, luciferase, and β -glucuronidase (GUS). Phosphinothricin acetyl transferase may be used as a selection marker in combination with the herbicide basta or bialaphos.

The transgenic plant of the invention may be prepared by methods known in the art. The transformation method used will
20 depend on the plant species to be transformed and can be selected from any of the transformation methods known in the art such as *Agrobacterium* mediated transformation (Zambryski et al., EMBO Journal 2, pp 2143-2150, 1993), particle bombardment, electroporation (Fromm et al. 1986, Nature 319, pp 791-793), and
25 virus mediated transformation. For transformation of monocots particle bombardment (ie biolistic transformation) of embryogenic cell lines or cultured embryos are preferred. Below, references are listed, which disclose various methods for transforming various plants: Rice (Cristou et al. 1991,
30 Bio/Technology 9, pp. 957-962), Maize (Gordon-Kamm et al. 1990, Plant Cell 2, pp. 603-618), Oat (Somers et al. 1992,

Bio/Technology 10, pp 1589-1594), Wheat (Vasil et al. 1991, Bio/Technology 10, pp. 667-674, Weeks et al. 1993, Plant Physiology 102, pp. 1077-1084) and Barley (Wan and Lemaux 1994, Plant Physiology 102, pp. 37-48, review Vasil 1994; Plant Mol. Biol. 25, pp 925-937).

More specifically, Agrobacterium mediated transformation is conveniently achieved as follows:

A vector system carrying the thermostable phytase is constructed. The vector system may comprise of one vector, but it can comprise of two vectors. In the case of two vectors the vector system is referred to as a binary vector system (Gynheung An et al.(1980), Binary Vectors, Plant Molecular Biology Manual A3, 1-19).

An Agrobacterium based plant transformation vector consists of replication origin(s) for both E.coli and Agrobacterium and a bacterial selection marker. A right and preferably also a left border from the Ti plasmid from Agrobacterium tumefaciens or from the Ri plasmid from Agrobacterium rhizogenes is necessary for the transformation of the plant. Between the borders the expression construct is placed which contains the thermostable phytase gene and appropriate regulatory sequences such as promotor and terminator sequences. Additionally, a selection gene e.g. the neomycin phosphotransferase type II (NPTII) gene from transposon Tn5 and a reporter gene such as the GUS (beta-glucuronidase) gene is cloned between the borders. A disarmed Agrobacterium strain harboring a helper plasmid containing the virulens genes is transformed with the above vector. The transformed Agrobacterium strain is then used for plant transformation.

The invention also relates to a method of preparing a transgenic plant capable of expressing a thermostable phytase,

said method comprising the steps of (i) isolating a nucleotide sequence encoding a thermostable phytase; (ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and (iii) transforming the selected host plant with the expression construct.

The above method in which "at least one" replaces "a," when used in relation to the thermostable phytase, is also within this invention.

10 This method is an essentially non-biological method.

Any plant may be a selected host plant. More specifically, the plant can be dicotyledonous or monocotyledonous, for short a dicot or a monocot. Of primary interest are such plants which are potential food or feed components. These plants may comprise 15 phytic acid. Examples of monocot plants are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g. wheat, oats, rye, barley, rice, sorghum and maize (corn).

Examples of dicot plants are legumes, such as lupins, pea, 20 bean and soybean, and cruciferous (family Brassicaceae), such as cauliflower, oil seed rape and the closely related model organism Arabidopsis thaliana.

Of particular interest are monocotyledonous plants, in particular crops or cereal plants such as wheat (Triticum, e.g. 25 aestivum), barley (Hordeum, e.g. vulgare), oats, rye, rice, sorghum and corn (Zea, e.g. mays).

Of further particular interest are dicotyledonous plants, such as those mentioned above.

In a preferred embodiment, the ancestral plant or host 30 plant is per se a desired feed ingredient.

Examples**Example 1****FYT-assay - for analyzing phytase enzyme preparations**

The phytase activity can be measured using the following assay:

5 10 μ l diluted enzyme samples (diluted in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5) are added into 250 μ l 5 mM sodium phytate (Sigma) in 0.1 M sodium acetate, 0.01 % Tween20, pH 5.5 (pH adjusted after dissolving the sodium phytate; the substrate is preheated) and incubated for 30 minutes at 37°C. The reaction
10 is stopped by adding 250 μ l 10 % TCA and free phosphate is measured by adding 500 μ l 7.3 g FeSO₄ in 100 ml molybdate reagent (2.5 g (NH₄)₆Mo₇O₂₄·4H₂O in 8 ml H₂SO₄ diluted to 250 ml). The absorbance at 750 nm is measured on 200 μ l samples in 96 well microtiter plates. Substrate and enzyme blanks are
15 included. A phosphate standard curve is also included (0-2 mM phosphate). 1 FYT equals the amount of enzyme that releases 1 μ mol phosphate/min at the given conditions. This assay is preferred for phytase enzyme preparations (when not in admixture with other feed ingredients).

20

Example 2**FTU assay - for analyzing phytase in admixture with feed ingredients**

One FTU is defined as the amount of enzym, which at stan-
25 dard conditions (37°C, pH 5,5; reaction time 60 minutes and start concentration of phytic acid 5 mM) releases phosphate equivalent to 1 μ mol phosphate per minute.

$$1 \text{ FTU} = 1 \text{ FYT}$$

The FTU assay is preferred for phytase activity measure-
30 ments on animal feed premixes and the like complex compositions.

Reagents /substratesExtraction buffer for feed etc.

This buffer is also used for preparation of PO_4 -standards and further dilution of premix samples.

5 0,22 M acetate buffer with Tween 20 pH 5,5

30 g sodium acetate trihydrate (MW = 136,08 g/mol) e.g. Merck Art 46267 per liter and 0,1 g Tween 20 e.g. Merck Art 22184 pr. liter are weighed out.

The sodium acetate is dissolved in demineralised water.

10 Tween 20 is added, and pH adjusted to $5,50 \pm 0,05$ with acetic acid.

Add demineralised water to total volume.

Extraction buffer for premix

0,22 M acetate buffer with Tween 20, EDTA, PO_4^{3-} og BSA.

15 30 g sodium acetate trihydrate e.g. Merck Art 6267 per liter.

0,1 g Tween 20 e.g. Merck Art 22184 per liter.

30 g EDTA f.eks. Merck Art 8418 pr. liter.

20 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ e.g. Merck Art 6580 per liter.

20 0,5 g BSA (Bovine Serum Albumine, e.g. Sigma Art A-9647 per liter.

The ingredients are dissolved in demineralised water, and pH is adjusted to $5,50 \pm 0,05$ with acetic acid.

Add demineralised water to total volume.

25 BSA is not stable, and must therefore be added the same day the buffer is used.

50 mM PO₄³⁻ stock solution

0,681 g KH₂PO₄ (MW = 136,09 g/mol) e.g. Merck Art 4873 is weighed out and dissolved in 100 ml 0,22 M sodium acetat with Tween, pH 5,5.

5 Storage stability: 1 week in refrigerator.

0,22 M acetate buffer pH 5,5 without Tween

This buffer is used for production of phytic acid substrate).

15 150 g sodium acetate trihydrate (MW = 136,08) e.g. Merck Art 6267 is weighed out and dissolved in demineralised water, and pH is adjusted with acetic acid to 5,50 ± 0,05.

Add demineralised water to 5000 ml.

Storage stability: 1 week at room temperature.

Phytic acid substrate: 5 mM phytic acid

15 The volume of phytic acid is calculated with allowance for the water content of the used batch.

If the water content is e.g. 8,4 % the following is obtained:

$$20 \frac{0,005 \text{ mol} / \text{l} \times 923,8 \text{ g} / \text{mol}}{(1 \div 0,084)} = 5,04 \text{ g} / \text{l}$$

Phytic acid (Na-salt) (MW = 923,8 g/mol) e.g. Sigma P-8810 is weighed out and dissolved in 0,22 M acetate buffer (without tween). Addition of (diluted) acetic acid increases the dissolution speed.

25 pH is adjusted to 5,50 ± 0,05 with acetic acid.

Add 0,22 M acetate buffer to total volume.

21.7 % nitric acid solution

For stop solution.

1 part concentrated (65%) nitric acid is mixed into 2 parts demineralised water.

Molybdate reagent

5 For stop solution.

100 g ammonium heptamolybdate tetrahydrate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ e.g. Merck Art 1182 is dissolved in demineralised water. 10 ml 25 % NH_3 is added.

Add demineralised water to 1 liter.

10 0,24 % Ammonium vanadate

Bought from fra Bie & Berntsen.

Molybdat/vanadat stop solution

1 part vanadate solution (0,24 % ammonium vanadate) + 1 part molybdate solution are mixed. 2 parts 21,7 % nitric acid solution are added.

The solution is prepared not more than 2 hours before use, and the bottle is wrapped in tinfoil.

Samples

Frozen samples are defrosted in a refrigerator overnight.

20 Sample size for feed samples: At least 70 g, preferably 100 g.

Feed samples

Choose a solution volume which allows addition of buffer corresponding to 10 times the sample weight, e.g. 100 g is dissolved in 1000 ml 0,22 M acetate buffer with Tween, see enclosure 1. Round up to nearest solution volume.

If the sample size is approx. 100 g all the sample is ground in a coffee grinder and subsequently placed in tared

beakers. The sample weight is noted. It is not necessary to grind not-pelleted samples. If a sample is too big to handle, it is sample split into parts of approx. 100 g.

Magnets are placed in the beakers and 0,22 M acetate 5 buffer with Tween is added.

The samples are extracted for 90 minutes.

After extraction the samples rest for 30 minuts to allow for the feed to sediment. A 5 ml sample is withdrawn with a pi-
pette. The sample is taken 2 - 5 cm under the surface of the so-
10 lution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

Premix samples

Choose a solution volume which allows addition of buffer
15 corresponding to 10 times the sample weight. Round up to nearest solution volume.

If the samples have been weighed (50 - 100 g) all of the sample is placed in tared beakers. The sample weight is noted.
If a sample is too big to handle, it is split into parts of ap-
20 prox. 100 g.

Magnets are placed in the beakers and 0,22 M acetate buffer with Tween, EDTA og PO_4^{3-} is added.

The samples are extracted for 60 minutes.

After extraction the samples rest for 30 minutes to allow
25 for the premix to sediment. A 5 ml sample is withdrawn with a pipette. The sample is taken 2 - 5 cm under the surface of the solution and placed in a centrifuge glass, which is covered by a lid.

The samples are centrifuged for 10 minutes at 4000 rpm.

Analysis

Extracts of feed samples are analysed directly.

Extracts of premix are diluted to approx. 1,5 FTU/g (A_{415}
5 (main sample) < 1,0).

0,22 M acetate buffer with Tween 20 is used for the dilution.

Main Samples

2 x 100 ml of the supernatant from the extracted and cen-
10 trifuged samples are placed in marked glass test tubes and a magnet is placed in each tube.

When all samples are ready they are placed on a water bath with stirring. Temperature: 37 °C.

3,0 ml substrate is added.

15 Incubation for exactly 60 minutes after addition of substrate.

The samples are taken off the water bath and 2,0 ml stop solution is added (exactly 60 minutes after addition of substrate).

20 The samples are stirred for 1 minute or longer.

Feed samples are centrifuged for 10 minutes at 4000 rpm (It is not necessary to centrifuge premix samples).

Blind samples

100 ml of the supernatant from the extracted and centri-
25 futed samples are placed in marked glass test tubes, and a magnet is placed in each tube.

2,0 ml stop solution is added to the samples.

3,0 ml substrate is added to the samples.

The samples are incubated for 60 minutes at room temperature.

The feed samples are centrifuged for 10 minutes at 4000 rpm (it is not necessary to centrifuge premix samples).

Standards

2 x 100 ml are taken from each of the 8 standards and also 4 x 100 ml 0,22 M acetate buffer (reagent blind).

A_{415} is measured on all samples.

10 CALCULATION

$$\text{FTU/g} = \mu\text{mol PO}_4^{3-} / (\text{min} * \text{g (sample)})$$

C g sample is weighed out (after grinding).

15 100 μl is taken from the extracted and centrifuged sample.

PO_4^{3-} standard curve is linear.

From the regression curve for the PO_4^{3-} standard the actual concentration of the sample is found (concentration in mM):

$$[\text{PO}_4^{3-}] = (x - b) / a \quad x = A_{415} \quad a = \text{slope} \quad b = \text{intercept with y-axis}$$

$$25 \quad \mu\text{mol PO}_4^{3-}/\text{min} = \{ [\text{PO}_4^{3-}] (\text{mM}) \times \text{Vol (liter)} \times 1000 \mu\text{mol/mmol} \} / t$$

t = incubation time in minutes.

Vol = sample volume in liter = 0,0001 liter

1000 = conversion factor from mmol to μmol

$$FTU / g_{\text{probe}} = \{ (x - b) \times Vol \times 1000 \times F_p \} / \{ a \times t \times C \}$$

C = gram sample weighed out

- 5 F_p = Relation between the sample taken out and the total sample (after extraction). Example: 0,100 ml taken from 1000 ml $\rightarrow F_p = 1000/0,100 = 10000$.

Reduced expression with insertion of the following values:

10 $t = 60$

$Vol = 0,0001 \text{ l}$

$F_p = 10000$

$FTU / g_{\text{sample}} = \{ (x - b) \times 0,0001 \times 1000 \times 10000 \} / \{ a \times 60 \times C \}$

15 Example 3

Determination of optimum temperature and melting point T_m of various phytases

The thermostability of various phytases has been determined, viz. the melting temperature, T_m , and/or the optimum
20 temperature.

The phytase of *Aspergillus niger* NRRL 3135 was prepared as described in EP 0420358 and van Hartingsveldt et al (Gene, 127, 87-94, 1993).

The phytases of *Aspergillus fumigatus* ATCC 13073,
25 *Aspergillus terreus* 9A-1, *Aspergillus terreus* CBS 116.46, *Aspergillus nidulans*, *Myceliophthora thermophila*, and *Talaromyces thermophilus* were prepared as described in EP-0897985 and the references therein.

Consensus-phytase-1 (Fig. 5) and Consensus-phytase-1-Q50T
30 are shown in and were prepared as described in EP 0897985.

Consensus-phytase-10 was derived and prepared according to the teachings of EP-0897985 (Examples 1-2 and 3-7, respectively), however adding to the alignment at Fig. 1 thereof the phytase sequence of *Thermomyces lanuginosa* (Berka et al, 5 Appl. Environ. Microbiol. 64, 4423-4427, 1998) and a basidiomycete consensus sequence (derivation described below), omitting the sequence of *A.niger* T213, and assigning a vote weight of 0.5 for the remaining *A.niger* phytase sequences. The derivation of the sequence of Consensus-phytase-10 is shown in 10 Fig. 7.

The basidiomycete consensus sequence was also derived according to the principles of EP-0897985, viz. from the five basidiomycete phytases of WO 98/28409, starting with the first amino acid residue of the mature phytases (excluding signal 15 peptide). A vote weight of 0.5 was assigned to the two *Paxillus* phytases, all other genes were used with a vote weight of 1.0 - see Fig. 6.

The muteins Consensus-phytase-10-thermo, Consensus-phytase-10-thermo-Q50T-K91A (Fig. 10) and Consensus-phytase-10-thermo-Q50T were prepared from consensus-phytase-10, in analogy 20 to Examples 5-8 of EP-0897985, by introducing the three back-mutations K94A, V158I and A396S ("thermo(3)" or "thermo") and, where applicable, also the mutations Q50T or Q50T-K91A.

The muteins Consensus-phytase-1-thermo(8), Consensus-phytase-1-thermo(8)-Q50T-K91A (Fig. 9) and Consensus-phytase-1-thermo(8)-Q50T, were prepared from consensus-phytase-1, in analogy to Example 8 of EP-0897985, by introducing the eight mutations E58A, D197N, E267D, R291I, R329H, S364T, A379K and G404A ("thermo(8)") and, where applicable, also the mutations 30 Q50T or Q50T-K91A.

Consensus-phytase-1-thermo(3) was prepared from consensus-phytase-1 by introduction of the three mutations K94A, V158I and A396S.

An *Aspergillus fumigatus* so-called α -mutant (with the 5 mutations Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D) and the further muteins thereof shown in Table 1 were prepared as generally described above. The position numbering refers to Fig. 11 hereof, except for the number in parentheses which refers to the numbering used in EP 0897010.

10 DNA constructs encoding the above thermostable phytases can be prepared e.g. according to the teachings of EP 0897985. For expression thereof in plants, reference is made to the present description.

In order to determine the unfolding temperature or melting 15 temperature, T_m , of a phytase, differential scanning calorimetry was applied as previously published by Brugger et al (1997): "Thermal denaturation of phytases and pH 2.5 acid phosphatase studied by differential scanning calorimetry," in The Biochemistry of phytate and phytase (eds. Rasmussen, S.K; Raboy, 20 V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers).

Homogenous or purified phytase solutions of 50-60 mg/ml of protein are prepared, and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min is applied up to 90-95°C.

25 The results of T_m determinations on the above phytases are shown in Table 1 below; for selected phytases also in Figs. 1-4.

In Table 1 below, the optimum temperature of various phytases is also indicated. For this determination, phytase activity was determined basically as described by Mitchell et al 30 (Microbiology 143, 245-252, 1997): The activity was measured in an assay mixture containing 0.5% phytic acid (~ 5 mM) in 200 mM

sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated phosphate was quantified by mixing 100 µl of the assay mixture with 900µl H₂O and 1 ml of 5 0.6 M H₂SO₄, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction 10 coefficient at 280 nm calculated according to Pace et al (Prot.Sci. 4, 2411-2423, 1995): Consensus phytase, 1.101; consensus phytase 7, 1.068; consensus phytase 10, 1.039.

For determination of the temperature optimum, enzyme (100µl) and substrate solution (100µl) were pre-incubated for 5 15 min at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min incubation, the reaction was stopped with trichloroacetic acid and the amount of phosphate released was determined. Phytase-activity-versus-temperature is plotted, and the temperature 20 optimum is determined as that temperature at which the activity reaches its maximum value.

Table 1

Temperature optimum and T_m for various phytases

25

Phytase	Optimum temperature (°C)	T _m (°C)
Aspergillus niger NRRL 3135	55	63.3
Aspergillus fumigatus ATCC 13073	55	62.5

	30	
Aspergillus terreus 9A-1	49	57.5
Aspergillus terreus CBS 116.46	45	58.5
Aspergillus nidulans	45	55.7
Myceliophthora thermophila	55	-
Talaromyces thermophilus	45	-
Consensus-phytase- 10-thermo-Q50T-K91A	82	89.3
Consensus-phytase- 10-thermo-Q50T	82	88.6
Consensus-phytase-10	80	85.4
Consensus-phytase-1- thermo (8) -Q50T-K91A	-	85.7
Consensus-phytase-1- thermo (8) -Q50T	78	84.7
Consensus-phytase-1- thermo (8)	81	-
Consensus-phytase-1- thermo (8) -Q50T-K91A	78	84.7
Consensus-phytase-1- thermo (3)	75	-
Consensus-phytase-1- Q50T	-	78.9
Consensus-phytase-1	71	78.1
Aspergillus fumigatus α -mutant, plus mutations E59A,	63	-

31

S126N, R329H, S364T, G404A		
Aspergillus fumigatus - as above, plus mutation K68A	63	-
Aspergillus fumigatus α -mutant (Q51(27)T, F55Y, V100I, F114Y, A243L, S265P, N294D)	60	67.0

CLAIMS

1. A process of preparing an animal feed, which process comprises an agglomeration of feed ingredients, wherein a thermostable phytase is added before or during the
5 agglomeration. -
2. The process of claim 1, wherein the feed ingredients are heated to a temperature of at least 65°C.
- 10 3. The process of any of claims 1-2, wherein the thermostable phytase is a phytase with a T_m as measured by DSC of at least 65°C, using for the DSC a constant heating rate of 10°C/min.
4. The process of any of claims 1-3, when performed in a feed
15 expander.
5. The process of any of claims 1-3, when performed in an extruder.
- 20 6. The process of any of claims 1-3, when performed in a pellet press.
7. The process of any of claims 1-6, wherein the thermostable phytase is present in a transgenic plant.
- 25 8. The process of any of claims 1-7, wherein the agglomeration includes the following steps:
 - (a) pre-heating the feed ingredients to a temperature of at least 45°C; and
 - 30 (b) heating the product of step (a) to a temperature of at least 65°C;

wherein the thermostable phytase is added prior to or during step (a) and/or (b).

9. A transgenic plant which comprises a DNA-construct
5 encoding a thermostable phytase.

10. The transgenic plant of claim 9, wherein the DNA-construct encoding the thermostable phytase is operably linked to regulatory sequences capable of mediating expression of said
10 phytase encoding sequence in at least one part of the plant.

11. An expression construct which comprises a DNA construct encoding a thermostable phytase, operably linked to regulatory sequences capable of mediating expression of said phytase
15 encoding sequence in at least one part of a plant.

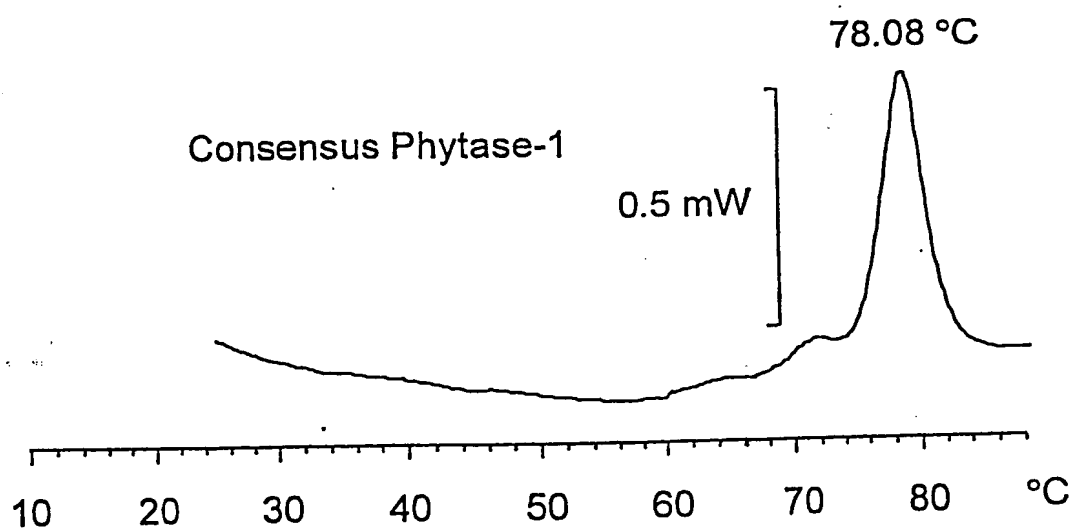
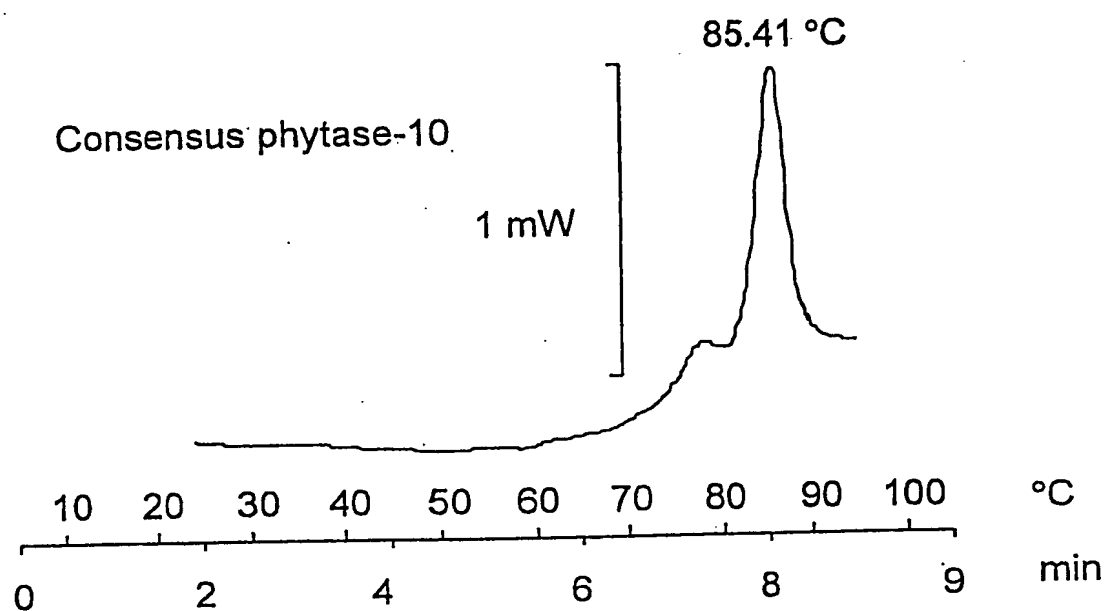
12. A vector which comprises the expression construct of claim 11.

20 13. A method of preparing a transgenic plant capable of expressing a thermostable phytase, said method comprising the steps of

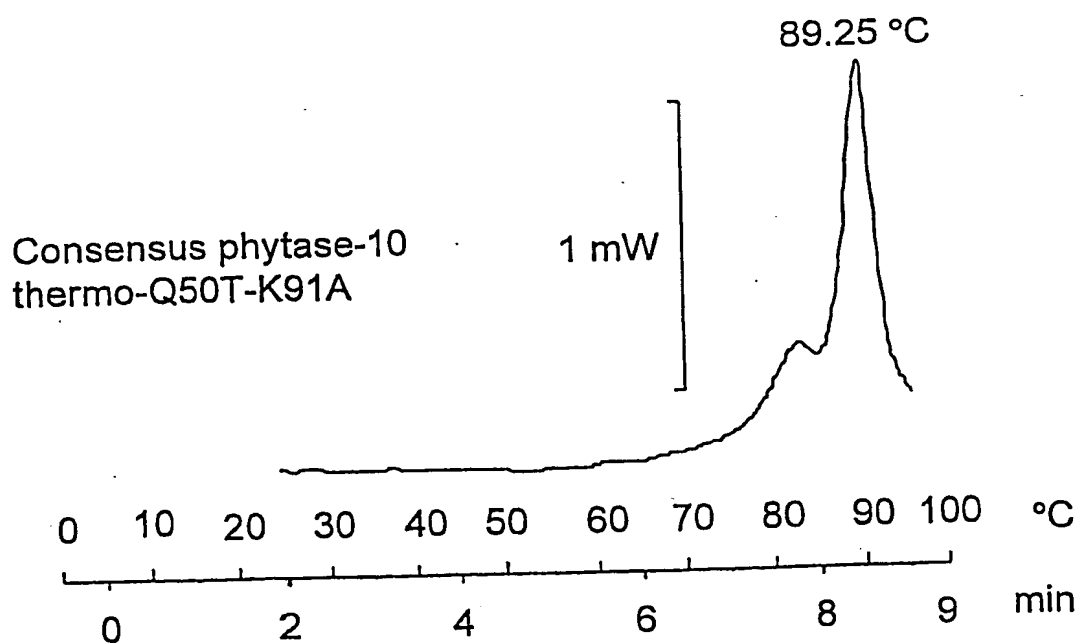
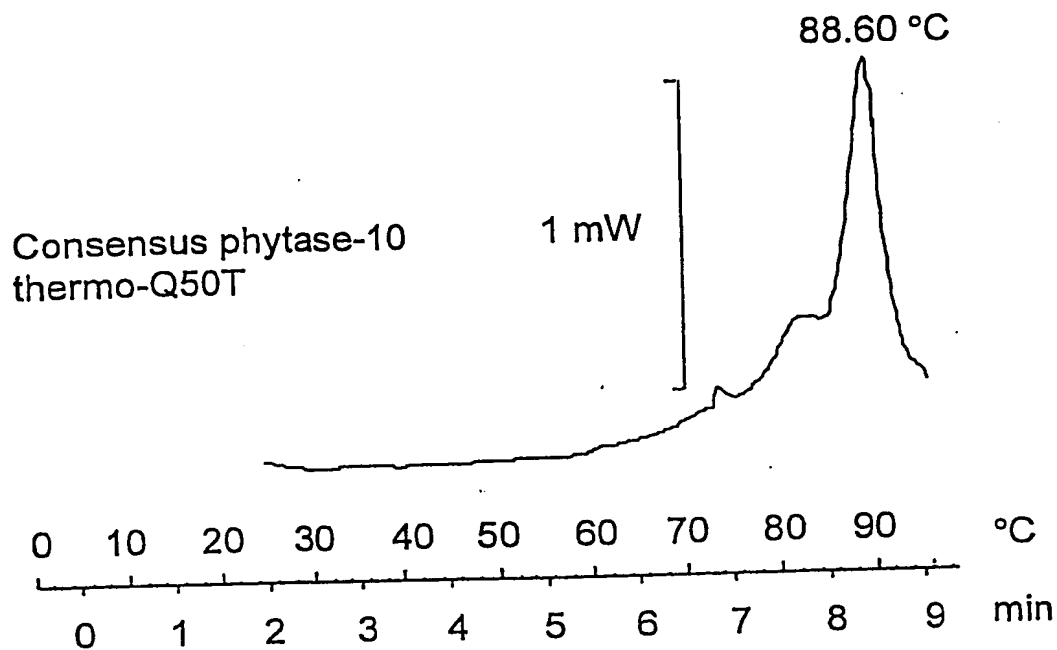
- (i) isolating a nucleotide sequence encoding a thermostable phytase;
- 25 (ii) inserting the nucleotide sequence of (i) in an expression construct capable of mediating the expression of the nucleotide sequence in a selected host plant; and
- (iii) transforming the selected host plant with the expression construct.

14. The method of claim 13, which comprises the further step of extracting the phytase from the plant.

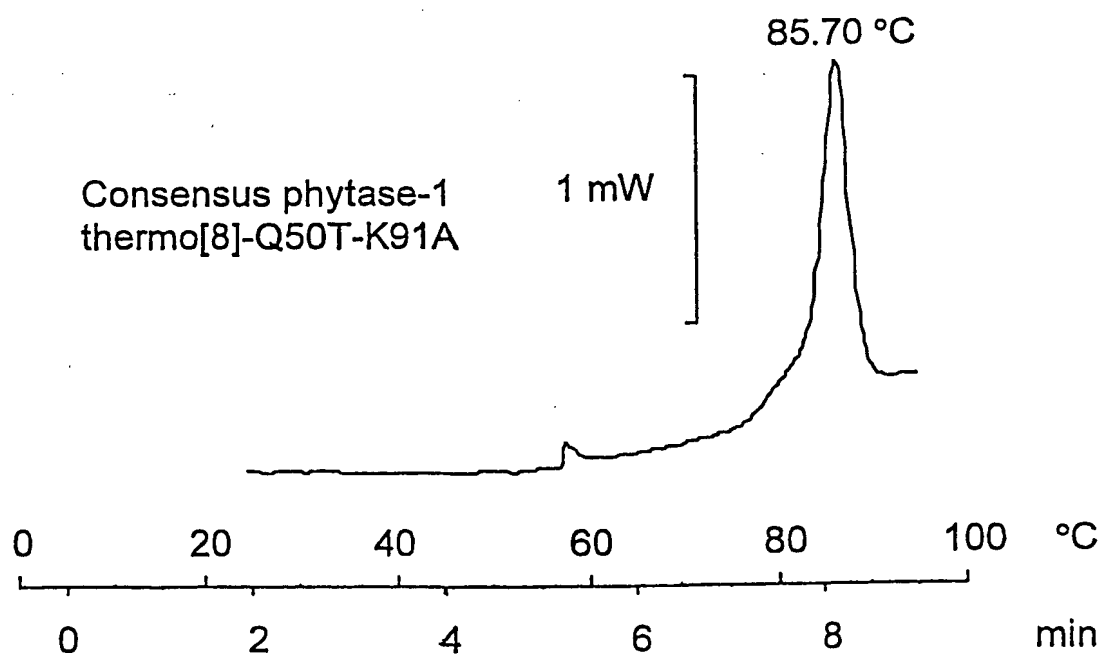
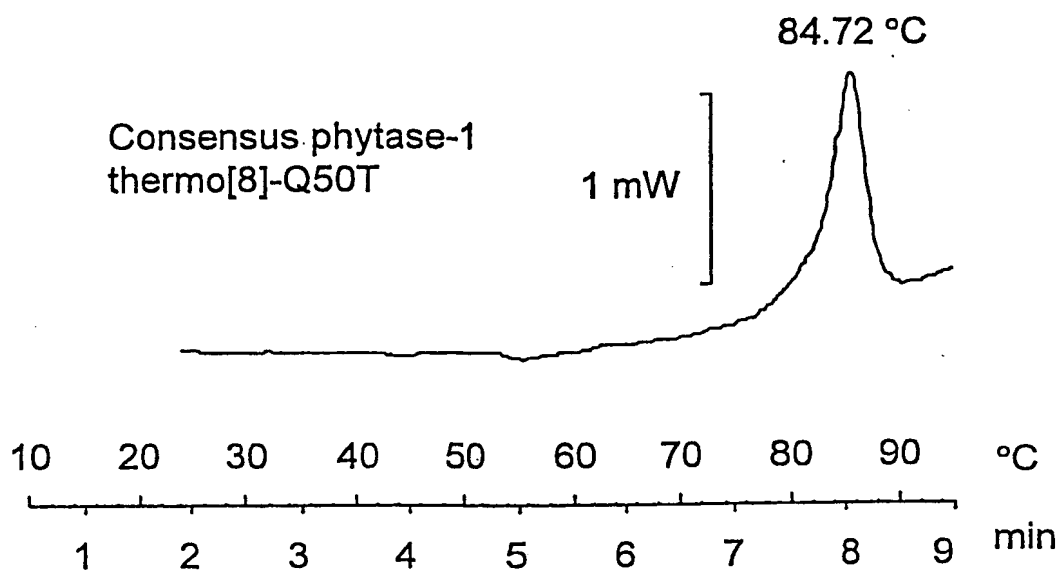
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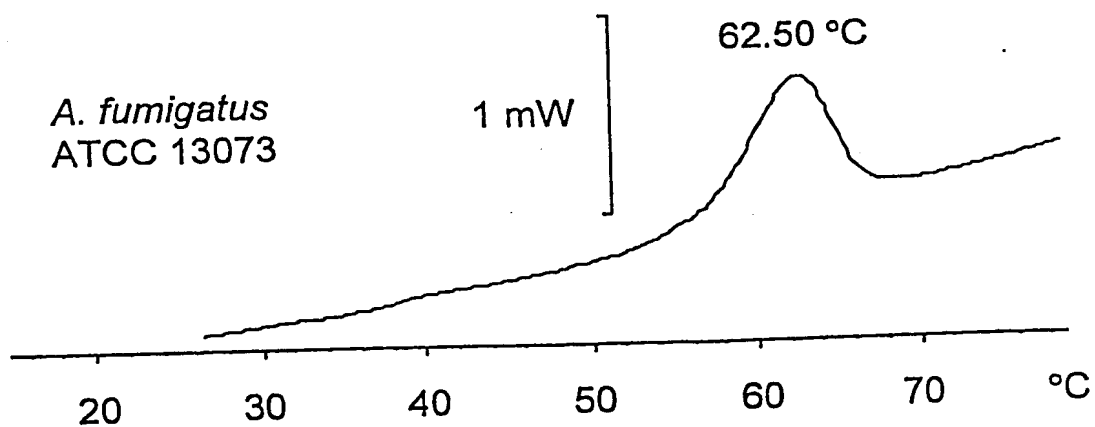
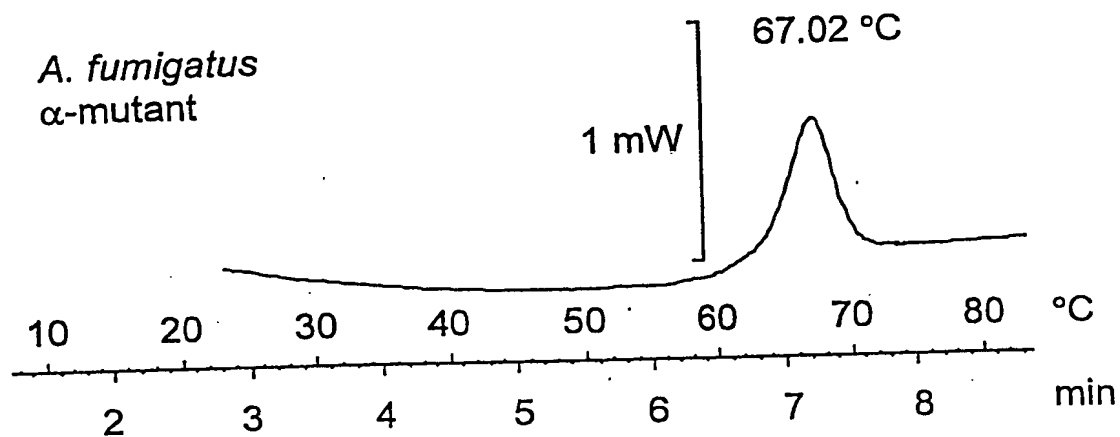
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1

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A. terreus 9A-1	KhsDCNSVDh	GYQCFPELSH	kWGLYAPYFS	LQDESFPFpLD	VPEDChITfV
A. terreus cbs	NhsDCTSVDr	GYQCFPELSH	kWGLYAPYFS	LQDESFPFpLD	VPDDChITfV
A. niger var. awamori	NqsTCDTVdQ	GYQCFSETSH	LGWQYAPFFS	LANESAISPD	VPAGCrVTfA
A. niger T213	NqsSCDTVDQ	GYQCFSETSH	LGWQYAPFFS	LANESVISPD	VPAGCrVTfA
A. niger NRRL3135	NqsSCDTVDQ	GYQCFSETSH	LGWQYAPFFS	LANESVISPE	VPAGCrVTfA
A. fumigatus 13073	GSKSCDTVDl	GYQCsPATSH	LGWQYSPFFS	LEDELSVSSK	LPKDCrITLV
A. fumigatus 32722	GSKSCDTVDl	GYQCsPATSH	LGWQYSPFFS	LEDELSVSSK	LPKDCrITLV
A. fumigatus 58128	GSKSCDTVDl	GYQCsPATSH	LGWQYSPFFS	LEDELSVSSK	LPKDCrITLV
A. fumigatus 26906	GSKSCDTVDl	GYQCsPATSH	LGWQYSPFFS	LEDELSVSSK	LPKDCrITLV
A. fumigatus 32239	GSKACDTVEL	GYQCsPGTSH	LGWQYSPFFS	LEDELSVSSD	LPKDCrVTfV
E. nidulans	QNHSCNTADG	GYQCFPNVSH	VWGQYSPYFS	IEQESAISeD	VPHGCeVTFV
T. thermophilus	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCkITfV
M. thermophila	ESRPCDTpDl	GFQCGTAISH	FWGQYSPYFS	VpSElDas..	IPDDCeVTFa
Consensus	NSHSCDTVDG	GYQCFPEISH	LGWQYSPYFS	LEDESAISPD	VPDDC-VTFV
Consensus phytase	NSHSCDTVDG	GYQCFPEISH	LGWQYSPYFS	LEDESAISPD	VPDDCrVTfV

51

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A. terreus 9A-1	QVLARHGARS	PTHSKtKAYA	AtIAAIQKSA	TaFpGKYAFL	QSYNYSLDSE
A. terreus cbs	QVLARHGARS	PTDSKtKAYA	AtIAAIQKNA	TaLpGKYAFL	KSYNYSMGSE
A. niger var. awamori	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTNYNSLGAD
A. niger T213	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTNYNSLGAD
A. niger NRRL3135	QVLSRHGARY	PTDSKgKkYS	ALIEEIQQNA	TtFDGKYAFL	KTNYNSLGAD
A. fumigatus 13073	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTNYNTLGAD
A. fumigatus 32722	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTNYNTLGAD
A. fumigatus 58128	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTNYNTLGAD
A. fumigatus 26906	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTNYNTLGAD
A. fumigatus 32239	QVLSRHGARY	PTASKsKkYK	kLVTAIQKNA	TeFKGKFAFL	ETNYNTLGAD
E. nidulans	QVLSRHGARY	PTESKsKAYS	GLIEAIQKNA	TaFwGQYAFI	ESYNYTLGAD
T. thermophilus	QVLSRHGARY	PTSSKtELYS	QLISrIQKTA	TaYKGyYAFL	KDYrYqLGAN
M. thermophila	QVLSRHGARA	PTIKRaaSYv	DLIDrIHhGA	IsYgPgYEFL	RTYDYTLGAD
Consensus	QVLSRHGARY	PTSSK-KAYS	ALIEAIQKNA	T-FKGKYAFL	KTNYNTLGAD
Consensus phytase	QVLSRHGARY	PTSSKSKAYS	ALIEAIQKNA	TAFKGKYAFL	KTNYNTLGAD

101

150

A. terreus 9A-1	ELTPFGrNQL	rDlGaQFYeR	YNALTrhInP	FVRATDASRV	hesAEKFVEG
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTLTrhInP	FVRAADSSRV	hesAEKFVEG
A. niger var. awamori	DLTPFGEQEL	VNSGIKfYQR	YESLTrNIIP	FIRSSGSSRV	IASGEKFIEG
A. niger T213	DLTPFGEQEL	VNSGIKfYQR	YESLTrNIIP	FIRSSGSSRV	IASGEKFIEG
A. niger NRRL3135	DLTPFGEQEL	VNSGIKfYQR	YESLTrNIVP	FIRSSGSSRV	IASGKfIEG
A. fumigatus 13073	DLTPFGEQQL	VNSGIKfYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 32722	DLTPFGEQQL	VNSGIKfYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 58128	DLTPFGEQQL	VNSGIKfYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 26906	DLTAFGEQQL	VNSGIKfYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
A. fumigatus 32239	DLTPFGEQQM	VNSGIKfYQK	YKALAgSVVP	FIRSSGSDRV	IASGEKFIEG
E. nidulans	DLTifGENQM	VDsGaKfYRR	YKNLARKnTP	FIRASGSDRV	VASAEKFING
T. thermophilus	DLTPFGENQM	IQlGIKfYnH	YKSLARNAvP	FVRCsGSDRV	IASGrIfIEG
M. thermophila	ELTrtGQQQM	VNSGIKfYRR	YRALARKsIP	FVRTAGqDRV	VhSAENFTQG
Consensus	DLTPFGENQM	VNSGIKfYRR	YKALARK-VP	FVRASGSDRV	IASAEKFIEG
Consensus phytase	DLTPFGENQM	VNSGIKfYRR	YKALARKIVP	FIRASGSDRV	IASAEKFIEG

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151

200

A. terreus 9A-1
A. terreus cbs
A. niger var. awamori
A. niger T213
A. niger NRRL3135
A. fumigatus 13073
A. fumigatus 32722
A. fumigatus 58128
A. fumigatus 26906
A. fumigatus 32239
E. nidulans
T. thermophilus
M. thermophila

FQTARqDDHh ANpHQSPPrV DVaIPEGSAY NNTLEHSICT AFES...STV
FQNAReGDPh ANpHQSPPrV DVVIEPGTAY NNTLEHSICT AFEA...STV
FQSTKLkDPr AqpGQSSPKI DVVIEASSs NNTLDPGTCT VFED...SEL
FQSTKLkDPr AqpGQSSPKI DVVIEASSs NNTLDPGTCT VFED...SEL
FQSTKLkDPr AqpGQSSPKI DVVIEASSs NNTLDPGTCT VFED...SEL
FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT KFEA...SQL
FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT KFEA...SQL
FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT KFEA...SQL
FQqAKLADPG A.TNRAAPAI SVIIPESETF NNTLDHGVCT KFEA...SQL
FQqANVADPG A.TNRAAPVI SVIIPESETY NNTLDHGVCT NFEA...SEL
FRKAQLhDHG S..GQATPVV NVIIPeIDGF NNTLDHSTCV SFEN...Der
FQSAKVLDPH SDkHDAPPTI NVIIeEGPSY NNTLDtGSCP VFED...SSg
FHSALLADRG STVRTPtPyd mVVIPETAGa NNTLHNDICT AFEEgpySTI

Consensus
Consensus phytase

FQSAKLADPG S-PHQASPVV NVIIPESGSY NNTLDHGTCT AFED---SEL
FQSAKLADPG SQPHQASPVV DVIIPEGSGY NNTLDHGTCT AFED...SEL

201

250

A. terreus 9A-1
A. terreus cbs
A. niger var. awamori
A. niger T213
A. niger NRRL3135
A. fumigatus 13073
A. fumigatus 32722
A. fumigatus 58128
A. fumigatus 26906
A. fumigatus 32239
E. nidulans
T. thermophilus
M. thermophila

GDDAVANFTA VFAPAIaQRL EADLPGVqLS TDDVVnLMAM CPFETVS1TD
GDAADNFTA VFAPAIaKRL EADLPGVqLS ADDVVnLMAM CPFETVS1TD
ADTVEANFTA TFAPSIRQRL ENDLsgvTLT DTEVtYLMdM CSFDTISTST
ADTVEANFTA TFAPSIRQRL ENDLsgvTLT DTEVtYLMdM CSFDTISTST
ADTVEANFTA TFAPSIRQRL ENDLsgvTLT DTEVtYLMdM CSFDTISTST
GDEVAANFTA lFAPDIRARA EkHLPgVtLT DEDVVslMDM CSFDTVARTS
GDEVAANFTA lFAPDIRARA EkHLPgVtLT DEDVVslMDM CSFDTVARTS
GDEVAANFTA lFAPDIRARA EkHLPgVtLT DEDVVslMDM CSFDTVARTS
GDEVAANFTA lFAPDIRARA KkHLPgVtLT DEDVVslMDM CSFDTVARTS
GDEVEANFTA lFAPAIRARI EkHLPgVqLT DDDVVslMDM CSFDTVARTS
ADEiEANFTA IMGPPIRkRL ENDLPGIKLT NENViYLMdM CSFDTMARTA
GHDAQEKFAK qFAPAIleKI KDHLPGVDLA vSDVpyLMdL CPFETLARNH
GDDAQDTYLS TFAGPitARV NANLPGANLT DADTVaLMdL CPFETVASSS

Consensus
Consensus phytase

GDDAEANFTA TFAPAIRARL EADLPGVtLT DEDVV-LMDM CPFETVARTS
GDDVEANFTA lFAPAIRARL EADLPGVtLT DEDVVYLMdM CPFETVARTS

251

300

A. terreus 9A-1
A. terreus cbs
A. niger var. awamori
A. niger T213
A. niger NRRL3135
A. fumigatus 13073
A. fumigatus 32722
A. fumigatus 58128
A. fumigatus 26906
A. fumigatus 32239
E. nidulans
T. thermophilus
M. thermophila

..... .DAhtLSPFC DLFTateWtq YNYLlSLDKY YGYGGGNPLG
..... .DAhtLSPFC DLFTaaEWtq YNYLlSLDKY YGYGGGNPLG
..... .vDTKLSPFC DLFTHdEWih YDYlQSLkKY YGHGAGNPLG
..... .vDTKLSPFC DLFTHdEWih YDYlRSLkKY YGHGAGNPLG
..... .vDTKLSPFC DLFTHdEWih YDYlQSLkKY YGHGAGNPLG
..... .DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
..... .DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
..... .DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
..... .DASQLSPFC QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
..... .DASELSPFC AIFTHnEWkk YDYlQSLGKY YGYGAGNPLG
..... .HGTELSPFC AIFTEkEWlq YDYlQSLSKY YGYGAGSPLG
..... .TDT.LSPFC ALstQeEWqa YDYlQSLGKY YGnGGGNPLG
sdpatadagg gNGrPLSPFC rLFSEsEWra YDYlQSVGKW YGYGPGNPLG

Consensus
Consensus phytase

----- .DATELSPFC ALFTE-EW-- YDYlQSLGKY YGYGAGNPLG
..... .DATELSPFC ALFTHdEWRO YDYlQSLGKY YGYGAGNPLG

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301

350

A. terreus 9A-1	PVQGVGWANE	LMARLTRAPV	HDHTCVNNTL	DASPATFFPLN	ATLYADFSHD
A. terreus cbs	PVQGVGWANE	LIARLTRSPV	HDHTCVNNTL	DANPATFFPLN	ATLYADFSHD
A. niger var. awamori	PTQGVGYANE	LIARLTHSPV	HDDTSSNHTL	DSNPATFFPLN	STLYADFSHD
A. niger T213	PTQGVGYANE	LIARLTHSPV	HDDTSSNHTL	DSNPATFFPLN	STLYADFSHD
A. niger NRRL3135	PTQGVGYANE	LIARLTHSPV	HDDTSSNHTL	DSSPATFFPLN	STLYADFSHD
A. fumigatus 13073	PAQGIGFTNE	LIARLTRSPV	QDHTSTNstL	vSNPATFFPLN	ATMYVDFSHD
A. fumigatus 32722	PAQGIGFTNE	LIARLTRSPV	QDHTSTNstL	vSNPATFFPLN	ATMYVDFSHD
A. fumigatus 58128	PAQGIGFTNE	LIARLTRSPV	QDHTSTNstL	vSNPATFFPLN	ATMYVDFSHD
A. fumigatus 26906	PAQGIGFTNE	LIARLTRSPV	QDHTSTNstL	vSNPATFFPLN	ATMYVDFSHD
A. fumigatus 32239	PAQGIGFTNE	LIARLTNSPV	QDHTSTNstL	DSDPATFFPLN	ATIYVDFSHD
E. nidulans	PAQGIGFTNE	LIARLTQSPV	QDHTSTNstL	DSNPATFFPLD	rKLYADFSHD
T. thermophilus	PAQGVGFvNE	LIARMTSPV	QDYTTVNHTL	DSNPATFFPLN	ATLYADFSHD
M. thermophila	PTQGVGFvNE	LLARLAGvPV	RDgTSTNRTL	DGDPtTFPLG	rPLYADFSHD
Consensus	PAQGVGF-NE	LIARLTHSPV	QDHTSTNHTL	DSNPATFFPLN	ATLYADFSHD
Consensus phytase	PAQGVGFANE	LIARLTRSPV	QDHTSTNHTL	DSNPATFFPLN	ATLYADFSHD

351

400

A. terreus 9A-1	SNLVSIFWAL	GLYNGTAPLS	qTSVESVSQT	DGYAAAWTVP	FAARAYVEMM
A. terreus cbs	SNLVSIFWAL	GLYNGTKPLS	qTTVEDITrT	DGYAAAWTVP	FAARAYIEMM
A. niger var. awamori	NGIISILFAL	GLYNGTKPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. niger T213	NGIISILFAL	GLYNGTKPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. niger NRRL3135	NGIISILFAL	GLYNGTKPLS	TTTVENITQT	DGFSSAWTVP	FASRLYVEMM
A. fumigatus 13073	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFETM
A. fumigatus 32722	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFETM
A. fumigatus 58128	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFETM
A. fumigatus 26906	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFETM
A. fumigatus 32239	NGMIPIFFAM	GLYNGTEPLS	qTSeESTKES	NGYSASWAVP	FGARAYFETM
E. nidulans	NSMISIFFAM	GLYNGTQPLS	mDSVESIQEm	DGYAASWTVP	FGARAYFELM
T. thermophilus	NTMTSIFaAL	GLYNGTAKLS	TTEIKSIEET	DGYSAAWTVP	FGGRAYIEMM
M. thermophila	NDMMGVLGAL	GayDGVPLD	KTArDpeEL	GGYAASWAVP	FAARLYVEKM
Consensus	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYAASWTVP	FGARAYVEMM
Consensus phytase	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYSASWTVP	FGARAYVEMM

401

450

A. terreus 9A-1	QC.....RAEKE	PLVRVLVNDR	VMPLHGCPD	KLGRCKrDAF
A. terreus cbs	QC.....RAEKQ	PLVRVLVNDR	VMPLHGCAVD	NLGRCKrDDF
A. niger var. awamori	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger T213	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger NRRL3135	QC.....QAEQE	PLVRVLVNDR	VVPLHGCPVD	aLGRCTrDSF
A. fumigatus 13073	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 32722	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 58128	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 26906	QC.....KSEKE	PLVRALINDR	VVPLHGCDVD	KLGRCKLNDF
A. fumigatus 32239	QC.....KSEKE	PLVRALINDR	VVPLHGCAVD	KLGRCKLKDF
E. nidulans	QC.....E.KKE	PLVRVLVNDR	VVPLHGCAVD	KFGRCTLDDW
T. thermophilus	QC.....DDSDE	PVVRVLVNDR	VVPLHGCEVD	SLGRCKrDDF
M. thermophila	RCsgggggggg	gggrQEKDE	emVRVLVNDR	VMTLkGCGAD	ErGMCTLErF
Consensus	QC-----	-----QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKLDDF
Consensus phytase	QC.....QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKRDDF

451

471

A. terreus 9A-1	VAGLSFAQAG	GNWADCF---	-
A. terreus cbs	VEGLSFARAG	GNWAECF---	-
A. niger var. awamori	VrGLSFARSG	GDWAECsA--	-
A. niger T213	VrGLSFARSG	GDWAECFA--	-
A. niger NRRL3135	VrGLSFARSG	GDWAECFA--	-
A. fumigatus 13073	VKGLSWARSG	GNWGECSF--	-
A. fumigatus 32722	VKGLSWARSG	GNWGECSF--	-
A. fumigatus 58128	VKGLSWARSG	GNWGECSF--	-
A. fumigatus 26906	VKGLSWARSG	GNWGECSF--	-
A. fumigatus 32239	VKGLSWARSG	GNSEQSFS--	-
E. nidulans	VEGLNFARSG	GNWkTCFTl-	-

8/32

<i>T. thermophilus</i>	VrGLSFARqG GNWEGCYAas e
<i>M. thermophila</i>	IESMAFARGN GKWDlCFA-- -
Consensus	VEGLSFARSG GNWAECA-- -
Consensus phytase	VEGLSFARSG GNWAECA.. .

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1 50
P. involutus (phyA1) SvP.KnTAPt FPIPeseQrn WSPYSPYFPL AeYkAPPAGC QInQVNIQR
P. involutus (phyA2) SvP.RniAPK FSIPeseQrn WSPYSPYFPL AeYkAPPAGC EInQVNIQR
T. pubescens hiPlRdTSAC LdVTrDvQqs WSmYSPYFPa AtYvAPPASC QInQVHIQR
A. pediades GgvvQaTfvQ pfFFPQiQds WAAyTPYYPV qaYtPPPkDC KItQVNIQR
P. lycii StQfsfvAAQ LPIPaQntsn WGPYdPFFPV EpYaAPPEGC tVtQVNIQR

Basidio S-P-R-TAAQ LPIP-Q-Q-- WSPYSPYFPV A-Y-APPAGC QI-QVNIQR

51 100
P. involutus (phyA1) HGARFPTSGA TTRIKAGLTK LQGvqnfTDA KFNfiksFky dLGnsDLVFPF
P. involutus (phyA2) HGARFPTSGA ATRIKAGLSK LQsvqnfTDP KFDfiksFty dLGtsDLVFPF
T. pubescens HGARFPTSGA AKRIQTAVAK LKAAsnyTDP lLAFvtNyTY sLGqDsLveL
A. pediades HGARFPTSGA GTRIQAaVvK LQSAktyTDP RLDFLTnyTY tLGhDDLVPF
P. lycii HGARWPTSGA rSRqvAAVAK IQmArpTDP KYEFLnDfvy kFGvADLLPF

Basidio HGARFPTSGA ATRIQAaVAK LQSA---TDP KLDFL-N-TY -LG-DDLVFPF

101 150
P. involutus (phyA1) GAaQSfDAGQ EAFARYSkLV SkNNLPFIRA dGSDRVVDSA TNWTAGFAsA
P. involutus (phyA2) GAaQSfDAGl EvFARYSkLV SsDNLFFIRS dGSDRVVDTA TNWTAGFAsA
T. pubescens GATQSSSEAGQ EAFTRYSSLV SaDELFPFVRA SGSDRVVATA nNWTAGFALA
A. pediades GALQSSQAGE ETfQRYsflV SkENLPFVRA SSSNRVVDSA TNWTEGFSaA
P. lycii GAnQShQTGt DmYTRYStLf egGDVPFVRA AGdQRVVDS TNWTAGFGdA

Basidio GA-QSSQAGQ EAFTRYs-LV S-DNLFPFVRA SGSDRVVDSA TNWTAGFA-A

151 200
P. involutus (phyA1) ShNTvqPkLn LILPQtGNDT LEDNMCPaAG DSDPQvNaWL AVafPSITAR
P. involutus (phyA2) SrNAiqPkLd LILPQtGNDT LEDNMCPaAG ESDPQvDaWL AsafPSVTAQ
T. pubescens SsNSitPvLs VIIEaGNDT LDDNMCPaAG DSDPQvNqWL AqFAPPMTAR
A. pediades ShHvlnPiLf VILSEsLNDT LDDaMCPnAG sSDPQtGiWt SIYGTPIAnR
P. lycii SgETvlpTLq VVLqEeGNCT LcNNMCPnEv DGDest.tWL GVFApNITAR

Basidio S-NT--P-L- VILSE-GNDT LDDNMCP-AG DSDPQ-N-WL AVFAPPITAR

201 250
P. involutus (phyA1) LNAAAPsvNL TDtDAfNLvs LCAFlTVSke kksdFctLFE giPGsFeAFa
P. involutus (phyA2) LNAAAPGANL TDaDAfNLvs LCPFmTVSke qksdFctLFE giPGsFeAFa
T. pubescens LNAGAPGANL TDtDTyNLlt LCPFETVatE rrSeFCDIYE elQAE.dAFa
A. pediades LNqqAPGANI TAAdvsNLip LCAFETivke tpSpFCNLf. .tPEEFaqFe
P. lycii LNAAAPSANL SDsDALtLmd MCPFDTLSSG naSpFCDLf. .tAEYvSYe

Basidio LNAAAPGANL TD-DA-NL-- LCPFETVS-E --S-FCDLFE --PEEF-AF-

251 300
P. involutus (phyA1) YgGDLDKfYG TGYGQeLGPV QGVGYVNELI ARLTnsAVRD NTQTNRtLDA
P. involutus (phyA2) YaGDLDKfYG TGYGQALGPV QGVGYINELL ARLTnsAVnD NTQTNRtLDA
T. pubescens YnADLDKfYG TGYGQPLGPV QGVGYINELI ARLTaQnVsD HTQTnStLDS
A. pediades YfGDLDKfYG TGYGQPLGPV QGVGYINELL ARLTempVRD NTQTNRtLDS
P. lycii YyyDLdKYyG TGpGNALGPV QGVGYVNEll ARLTgQAVRD ETQTNRtLDS

Basidio Y-GDLDKfYG TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRtLDS

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<i>P. involutus</i> (phyA1)	SPvTFPLNKT	FYADFSHDN1	MVAVFSAMGL	FrQPAPLsTS	vPNPwRTWzT
<i>P. involutus</i> (phyA2)	APdTFPLNKT	MYADFSHDN1	MVAVFSAMGL	FrQSAPLsTS	tPDPNRTWLT
<i>T. pubescens</i>	SPeTFPLNRT	LYADFSHDNQ	MVAIFSAMGL	FNQSAPLDPT	tPDPaRTFLv
<i>A. pediades</i>	SPlTFPLDRS	IYADLSHDNQ	MIAIFSAMGL	FNQSSPLDPS	fPNPKRTWVT
<i>P. lycii</i>	dPaTFPLNRT	FYADFSHDNt	MVPIFAALGL	FNAtA.LDP1	kPDenR1WVd

Basidio	SP-TFPLNRT	FYADFSHDNQ	MVAIFSAMGL	FNQSAPLDPS	-PDPNRTWVT
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	351		400
<i>P. involutus</i> (phyA1)	SsLVPFSGRM	VVERLsC..f	GT.....tkV RVLVQDqVQP
<i>P. involutus</i> (phyA2)	SsVVPFSARM	aVERLsC..a	GT.....tkV RVLVQDqVQP
<i>T. pubescens</i>	kKIVPFSGRM	VVERLdC..g	GA.....qsV RLLVNDAVQP
<i>A. pediades</i>	SRLtPFSGRM	VtERLlCqrd	GTgsgggsri mringnvqtfv RILVNDAVQP
<i>P. lycii</i>	SKLVPFSGHM	tVEKLsC...sgkeaV RVLVNDAVQP

Basidio	SKLVPFSGRM	VVERL-C---	GT-----V RVLVNDAVQP
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	401		441
<i>P. involutus</i> (phyA1)	LEFCGGDrNG	lCTLakFVES	QtFARsDGaG DFEKCFATSa -
<i>P. involutus</i> (phyA2)	LEFCGGDqDG	lCALDkFVES	QaYARsGGaG DFEKCLATTv -
<i>T. pubescens</i>	LAFCGADtsG	vCTLDAFVES	QaYARNDGEG DFEKCFAT-- -
<i>A. pediades</i>	LKFCGGDmDS	lCTLEAFVES	QkYAREDGQG DFEKCFD--- -
<i>P. lycii</i>	LEFCGG.vDG	vCeLsAFVES	QtYARENGQG DfAKCgfvPs e

Basidio	LEFCGGD-DG	-CTLDAFVES	Q-YAREDGQG DFEKCFATP- -
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11/32

50

	1								
A. terreus 9a1	KhSDCNSVDh	GYQcfPELSH	kWGLYAPYFS	LqDESPPF1D	VPdDCHITFV				
A. terreus cbs	NhSDCtSVDr	GYQcfPELSH	kWGLYAPYFS	LqDESPPF1D	VPdDCHITFV				
A. niger var. awamori	NqSTCDTVDq	GYQcfSEtSH	LGWQYAPFFS	LANESAISPD	VPaGCVTFa				
A. niger NRRL3135	NqSSCDTVDq	GYQcfSEtSH	LGWQYAPFFS	LANESvISPE	VPaGCVTFa				
A. fumigatus 13073	GSKSCDTVD1	GYQCsPATSH	LGWQYSPFFS	LEDELsvSSK	LPkDCHITLV				
A. fumigatus 32722	GSKSCDTVD1	GYQCsPATSH	LGWQYSPFFS	LEDELsvSSK	LPkDCHITLV				
A. fumigatus 58128	GSKSCDTVD1	GYQCsPATSH	LGWQYSPFFS	LEDELsvSSK	LPkDCHITLV				
A. fumigatus 26906	GSKSCDTVD1	GYQCsPATSH	LGWQYSPFFS	LEDELsvSSK	LPkDCHITLV				
A. fumigatus 32239	GSKACDTVEL	GYQCsPGtSH	LGWQYSPFFS	LEDELsvSSD	LPkDCHITLV				
E. nidulans	QNHSCNTaDG	GYQcfPNVSH	VWGQYSPYFS	IEQESAISD	VPhGQeVTFV				
T. thermophilus	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPqNCKITFV				
T. lanuginosa	-----	nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPkGCRVeFV				
M. thermophila	ESRPCDTpD1	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPdDceVTFa				
Basidio	xSxPxrxTAA	qLPipxQxqx	xWSPYSPYFP	VAXyxA....	pPaGQIXqV				

Consensus NSHSCDTVDG GYQC-PEISH LGWQYSPFFS LADESAISPD VP-GCRVTFV
 Fcp10 NSHSCDTVDG GYQCfPEISH LGWQYSPFFS LADESAISPD VPKGCRVTFV

100

	51								
A. terreus 9a1	QVLARHGARS	PTHSKTKaYA	AtIaAIQKSA	TaFpGKYAFL	QSYNYSLDSE				
A. terreus cbs	QVLARHGARS	PTdSKTKaYA	AtIaAIQKNA	TaLpGKYAFL	KSYNYSMGSE				
A. niger var. awamori	QVLSRHGARY	PTeSKGKKYS	ALIEEIQQNv	TtFDGKYAFL	KTYNYSLGAD				
A. niger NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIEEIQQNA	TtFDGKYAFL	KTYNYSLGAD				
A. fumigatus 13073	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD				
A. fumigatus 32722	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD				
A. fumigatus 58128	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD				
A. fumigatus 26906	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD				
A. fumigatus 32239	QVLSRHGARY	PTASKSKKYk	kLVtAIQKNA	TeFKGKFAFL	ETNYNYTLGAD				
E. nidulans	QVLSRHGARY	PTeSKSKaYS	GLIEAIQKNA	TsFwQYAFI	ESYNYTLGAD				
T. thermophilus	QLLSRHGARY	PTSSKTELYS	qLIsrIQKtA	TaYKGyYAFI	KdYrYqLGAN				
T. lanuginosa	QVLSRHGARY	PTAhKSEvYA	ELLqRIQDtA	TeFKGDFAFL	RdYaYhLGAD				
M. thermophila	QVLSRHGARA	PTlkRAAsYv	DLIdrIHhGA	isYgPgYEFL	RTYDYTLGAD				
Basidio	NIIqRHGARF	PTSGaAtRiq	AaVakLQsax	xxTDPKLDLFL	xxntYxLGxX				

Consensus QVLSRHGARY PTSSKSKKYs ALI-AIQKNA T-FKGKYAFL KTYNYTLGAD
 Fcp10 QVLSRHGARY PTSSKSKKYs ALIEAIQKNA TAFKGKYAFL KTYNYTLGAD

150

	101								
A. terreus 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRhIn	PFVRATDAsR	VhESAeKFVE				
A. terreus cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRhIn	PFVRAADSsR	VhESAeKFVE				
A. niger var. awamori	DLTPFGEQEL	VNSGIKFYQR	YESL.TrnII	PFIRSSGSsR	VIASGEKFIE				
A. niger NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TrnIV	PFIRSSGSsR	VIASGKKFIE				
A. fumigatus 13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE				
A. fumigatus 32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE				
A. fumigatus 58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE				
A. fumigatus 26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE				
A. fumigatus 32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgsVV	PFIRSSGSsR	VIASGEKFIE				
E. nidulans	DLTiFGENQM	VDSGaKFYRR	YKnL.Arknt	PFIRASGSDR	VVASAEKFIE				
T. thermophilus	DLTPFGENQM	IQlGIKFYnH	YKSL.ARnaV	PFVRCGSDR	VIASGrIFIE				
T. lanuginosa	NLTRFGEEQM	MESGrQFYHR	YREq.AReIV	PFVRAAGSAR	VIASAEffnR				
M. thermophila	ELTRtGQQQM	VNSGIKFYRR	YRAL.ARksI	PFVRTAGqDR	VVhSAENftQ				
Basidio	DLvPFGAxQs	sQAGqEaFtR	YsxLvSxdnL	PFVRASGSDR	VVDSACNWtA				

Consensus DLTPFGEQQM VNSGIKFYRR YKAL-AR-IV PFVRASGSDR VIASAEKFIE

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Fcp10 DLTPFGEQQM VNSGIKFYRR YKAL.ARKIV PFVRASGSDR VIASAEKPIE

	151		200
A. terreus 9a1	GFQTARqDDh	hAnphQPSPr	VDVaIPEGsA YNNTLEHSLC TAFEs...St
A. terreus cbs	GFQNArqGDP	hAnphQPSPr	VDVVIPEGtA YNNTLEHSIC TAFEa...St
A. niger var. awamori	GFQSTKLkDP	rAqpgQSSPk	IDVVIPEGsA sNNTLDpGtC TvFEd...SE
A. niger NRRL3135	GFQSTKLkDP	rAqpgQSSPk	IDVVIPEGsA sNNTLDpGtC TvFEd...SE
A. fumigatus 13073	GFQqAKLADP	gAt.nRAAPa	ISVVIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32722	GFQqAKLADP	gAt.nRAAPa	ISVVIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 58128	GFQqAKLADP	gAt.nRAAPa	ISVVIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 26906	GFQqAKLADP	gAt.nRAAPa	ISVVIPESeT FNNTLDHGVC TkFEa...SQ
A. fumigatus 32239	GFQqANVADP	gAt.nRAAPV	ISVVIPESeT YNNTLDHSVC TnFEa...SE
E. nidulans	GFRKAQLhDh	g.s.gQATPV	VNVIPEIdG FNNTLDHStC vSFEn...dE
T. thermophilus	GFQSAKVlDP	hSdKhDAPPT	INVIIeEGpS YNNTLDtGSc PvFEd...Ss
T. lanuginosa	GFQdAKdrDP	rSnkdQAePV	INVIISEEtG sNNTLDgtC PaaEe...Ap
M. thermophila	GFHSAIlADR	gStvrPTlPy	dmVVIPETaG aNNTLHNDLC TAFEegPySt
Basidio	GFaxA.....	..sXntxxPx	LxVILSExg. .NDTLDDNMCPxAG

Consensus	GFQSAKLADP	-A---QASPV	INVIIPEG-G	YNNTLDHGLC	TAFE--P-SE
Fcp10	GFQSAKLADP	GANPHQASPV	INVIIPEGAG	YNNTLDHGLC	TAFEE...SE

	201		250
A. terreus 9a1	VGDDaVANFT	AVFAPAIaQR	LEAdLPGVQL StDDVVNLMA MCPFETVSlT
A. terreus cbs	VGDAaADNFT	AVFAPAIaQR	LEAdLPGVQL SADDVVNLMA MCPFETVSlT
A. niger var. awamori	LADtVEANFT	AtFAPSIRqR	LEndLSGVtL TDteVtyLMD MCSFDtISTs
A. niger NRRL3135	LADtVEANFT	AtFvPSIRqR	LEndLSGVtL TDteVtyLMD MCSFDtISTs
A. fumigatus 13073	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 32722	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 58128	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 26906	LGDEVAANFT	ALFAPdIRAR	aKkhLPGVtL TDEDVVS LMD MCSFDtVArT
A. fumigatus 32239	LGDEVEANFT	ALFAPAIRAR	IEkhLPGVQL TDDDVVS LMD MCSFDtVArT
E. nidulans	rADEIEANFT	AIMGPPIRkR	LEndLPGIKL TNENViYlMD MCSFDtMArT
T. thermophilus	gGHDAQEKFA	kqFAPAIIEK	IKDhLPGVDL AvsDVpyLMD LCPFETLArN
T. lanuginosa	.DptqpAEFl	qVFGPRVlKk	ItkhMPGVNL TLEDVplFMD LCPFDtVGsd
M. thermophila	IGDDAQDtYl	StFAGPITAR	VNAnLPGaNL TDADtValMD LCPFETVAsS
Basidio	dSDpqxnXWl	AVFAPPITAR	LNAaapGaNL TDxDaxNLxx LCPFETVS..

Consensus	LGDDVEANFT	AVFAPPIRAR	LEA-LPGVNL	TDEDVVNLMD	MCPFDtVA-T
Fcp10	LGDDVEANFT	AVFAPPIRAR	LEAHLPGVNL	TDEDVVNLMD	MCPFDtVArT

	251		300
A. terreus 9a1	dD..Aht...LSPF	CDLFTa..tE WtQYNYLlSL dKYYGYGGGN
A. terreus cbs	dD..Aht...LSPF	CDLFTa..aE WtQYNYLlSL dKYYGYGGGN
A. niger var. awamori	Tv..DTK...LSPF	CDLFTH..dE WiHYDYlQSL kKYYGHGAGN
A. niger NRRL3135	Tv..DTK...LSPF	CDLFTH..dE WiNYDYlQSL kKYYGHGAGN
A. fumigatus 13073	SD..ASQ...LSPF	CQLFTH..nE WkKYNlQSL gKYYGYGAGN
A. fumigatus 32722	SD..ASQ...LSPF	CQLFTH..nE WkKYNlQSL gKYYGYGAGN
A. fumigatus 58128	SD..ASQ...LSPF	CQLFTH..nE WkKYNlQSL gKYYGYGAGN
A. fumigatus 26906	SD..ASQ...LSPF	CQLFTH..nE WkKYNlQSL gKYYGYGAGN
A. fumigatus 32239	AD..ASE...LSPF	CAIFTH..nE WkKYDYlQSL gKYYGYGAGN
E. nidulans	AH..GTE...LSPF	CAIFTE..kE WlQYDYlQSL sKYYGYGAGS
T. thermophilus	ht..DT....LSPF	CALStQ..eE WqayDYlQSL gKYYGnGGGN
T. lanuginosa	PvlfPrQ...LSPF	CHLFTa..dD WmayDYlQSL dKYYSHGGGS
M. thermophila	SsdpaTadag	ggnggrLSPF	CrLFSE..sE WrayDYlQSV gKYYGYGPGN
BasidioxexxSx	CDLFexxpeE FxaFxyxgdl dKfYgtGyGQ

Consensus	SD--ATQ---	-----LSPF	CDLFTH---E	W-QYDYlQSL	-KYYGYGAGN
Fcp10	SD..ATQ...LSPF	CDLFTH..DE	WiQYDYlQSL	gKYYGYGAGN

	301		350
A. terreus 9a1	PLGPvQGVGW	aNELMARLTr	A.PVHDHTCv NNTLDASPAT FPLNATLYAD
A. terreus cbs	PLGPvQGVGW	aNELIARLTr	S.PVHDHTCv NNTLDANPAT FPLNATLYAD
A. niger var. awamori	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS NHTLDSNPAT FPLNSTLYAD
A. niger NRRL3135	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS NHTLDSSPAT FPLNSTLYAD

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<i>A. fumigatus</i> 13073	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
<i>A. fumigatus</i> 32722	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
<i>A. fumigatus</i> 58128	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
<i>A. fumigatus</i> 26906	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT	FPLNATMYvD
<i>A. fumigatus</i> 32239	PLGPAQGIGF	tNELIARLTN	S.PVQDHTST	NsTLSDPAT	FPLNATIYvD
<i>E. nidulans</i>	PLGPAQGIGF	tNELIARLTQ	S.PVQDNTST	NHTLDSNPAT	FPLDkLYAD
<i>T. thermophilus</i>	PLGPAQGVGF	vNELIARMTg	S.PVQDYTTv	NHTLDSNPAT	FPLNATLYAD
<i>T. lanuginosa</i>	AFGSPRGVGF	vNELIARMTg	NLPVKDHTTv	NHTLDdNPET	FPLDAvLYAD
<i>M. thermophila</i>	PLGPTQGVGF	vNELIARLA.	GvPVRDgTST	NRTLGDGPtT	FPLGrPLYAD
Basidio	PLGPvQGVGY	iNELIARLTx	qa.VRDNTqT	NRTLDSsPxT	FPLNrTFYAD

Consensus	PLGPAQGVGF	-NELIARLTH	S-PVQDHTST	NHTLDSNPAT	FPLNATLYAD
Fcp10	PLGPAQGVGF	VNELIARLTH	S.PVQDHTST	NHTLDSNPAT	FPLNATLYAD

	351		400
<i>A. terreus</i> 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqtSVE. .SvsQTDGYA AAWTVPFAR
<i>A. terreus</i> cbs	FSHDSnLVSI	FWALGLYNGT	kPLSqtTVE. .ditrTDGYA AAWTVPFAR
<i>A. niger</i> var. <i>awamori</i>	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE. .NitQTDGFS SAWTVPFASR
<i>A. niger</i> NRRL3135	FSHDNGIISI	LFALGLYNGT	kPLSTTTVE. .NitQTDGFS SAWTVPFASR
<i>A. fumigatus</i> 13073	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKELDGYS ASWvVPFGAR
<i>A. fumigatus</i> 32722	FSHDNSMVSI	FFALGLYNGT	gPLSrTSVE. .SaKELDGYS ASWvVPFGAR
<i>A. fumigatus</i> 58128	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKELDGYS ASWvVPFGAR
<i>A. fumigatus</i> 26906	FSHDNSMVSI	FFALGLYNGT	ePLSrTSVE. .SaKELDGYS ASWvVPFGAR
<i>A. fumigatus</i> 32239	FSHDNGMIPI	FFAMGLYNGT	ePLSqtSeE. .StKESNGYS ASWAVPFGAR
<i>E. nidulans</i>	FSHDNSMISI	FFAMGLYNGT	qPLSmdSVE. .SiQEmDGYA ASWTVPFAR
<i>T. thermophilus</i>	FSHDNTMTSI	FaALGLYNGT	akLSTTeIK. .SiEETDGYA AAWTVPFGR
<i>T. lanuginosa</i>	FSHDNTMTGI	FsAMGLYNGT	kPLSTSkIQP pTgAAADGYA ASWTVPFAR
<i>M. thermophila</i>	FSHDNDMMGV	LgALGaYDGV	pPLdkTA..R rdpEELGGYA ASWAVPFAR
Basidio	FSHDNqMVAI	FsAMGLFNqS	aPLdPSxpDP nrt.....Wv TsklVPFSAR

Consensus	FSHDNTMVSI	FFALGLYNGT	-PLSTTSVEP	-S-EETDGYA	ASWTVPFAR
Fcp10	FSHDNTMVSI	FFALGLYNGT	KPLSTTSVE.	.SiEETDGYA	ASWTVPFAR

	401		450
<i>A. terreus</i> 9a1	AYVEMMQC..	ra.....EKEPL VRVLVNDRVm PLHGCPtDKL
<i>A. terreus</i> cbs	AYIEMMQC..	ra.....EKQPL VRVLVNDRVm PLHGCAVDNL
<i>A. niger</i> var. <i>awamori</i>	lyVEMMQC..	Qa.....EQEPL VRVLVNDRVV PLHGCPIDaL
<i>A. niger</i> NRRL3135	lyVEMMQC..	Qa.....EQEPL VRVLVNDRVV PLHGCPVdaL
<i>A. fumigatus</i> 13073	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32722	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 58128	AYfEtMQC..	Ks.....EKESL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 26906	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32239	AYfEtMQC..	Ks.....EKEPL VRaLINDRVV PLHGCAVDKL
<i>E. nidulans</i>	AYfELMQC..	E.....KKEPL VRVLVNDRVV PLHGCAVDKF
<i>T. thermophilus</i>	AYIEMMQC..	Dd.....sDEPV VRVLVNDRVV PLHGCEVDsL
<i>T. lanuginosa</i>	AYVELLRC..	Etetsseeee	EG...EDEPF VRVLVNDRVV PLHGCrVDRW
<i>M. thermophila</i>	iYVEkMRC..	sgggggggggg	EGrqeKDEEm VRVLVNDRVm TLkGCGaDer
Basidio	mvVErLxCxx	xgtxxxxxxx	xxxxxxxxxxx VRVLVNDaVq PLEfCGgDxd

Consensus	AYVEMMQC--	E-----	EG---EKEPL	VRVLVNDRVV	PLHGCGVDKL
Fcp10	AYVEMMQC..	EA.....EKEPL	VRVLVNDRVV	PLHGCGVDKL

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	451	482
A. terreus 9a1	GRCKrDAFVA GLSFAQAG..	GNWADCF--- --
A. terreus cbs	GRCKrDDFVE GLSFARAG..	GNWAECE--- --
A. niger var. awamori	GRCtrDsFVr GLSFARSG..	GDWAECsA-- --
A. niger NRRL3135	GRCtrDsFVr GLSFARSG..	GDWAECFA-- --
A. fumigatus 13073	GRCKlNDFVK GLSWARSG..	GNWGECEFS-- --
A. fumigatus 32722	GRCKlNDFVK GLSWARSG..	GNWGECEFS-- --
A. fumigatus 58128	GRCKlNDFVK GLSWARSG..	GNWGECEFS-- --
A. fumigatus 26906	GRCKlNDFVK GLSWARSG..	GNWGECEFS-- --
A. fumigatus 32239	GRCKlKDFVK GLSWARSG..	GNSEQSFS-- --
E. nidulans	GRCtlDDWVE GLNFARSG..	GNWktCFTl~ --
T. thermophilus	GRCKrDDFVr GLSFARqG..	GNWEGCYAas e-
T. lanuginosa	GRCRrDEWIK GLTFARqG..	GHWDrCF--- --
M. thermophila	GmCtlErFIE SMAFARGN..	GKWDlCFA-- --
Basidio	GxCtlDAFVE SqxYAReDgq	GDFEKCFAtp xx
Consensus	GRCK-DDFVE GLSFARSG--	GNWEECFa-- --
Fcp10	GRCKRDDFVE GLSFARSG..	GNWEECFa.. ..

15/32

	1		50
<i>P. involutus</i> (phyA1)	-----	-FPipeseqR nWSPYSPYFP LAEyKA....	pPaSCQInqV
<i>P. involutus</i> (phyA2)	-----	-FsipeseqR nWSPYSPYFP LAEyKA....	pPaSCeInqV
<i>T. pubescens</i>	-----	-LDvtRDVqQ sWSmYSPYFP aAtyvA....	pPaSCQInqV
<i>A. pediades</i>	-----	-pffpPQIQD sWAAyTPYYP VqAyTP....	pPKCKITqV
<i>P. lycii</i>	-----	-LPipAQnTs nWGPYdPFFP VEpyAA....	pPEECtVTqV
<i>A. terreus</i> 9a1	KhsdCNSVDh	GYQCfPELSH kWGLYAPYFS LqDESPFP1D	VPEDCHITFV
<i>A. terreus</i> cbs	NhsdCtSVDr	GYQCfPELSH kWGLYAPYFS LqDESPFP1D	VPEDCHITFV
<i>A. niger</i> var. <i>awamori</i>	NqstCDTVdQ	GYQCfSETSH LWGQYAPFFS LANESAISPD	VPaGCRVTFa
<i>A. niger</i> T213	NqssCDTVdQ	GYQCfSETSH LWGQYAPFFS LANESvISPD	VPaGCRVTFa
<i>A. niger</i> NRRL3135	NqssCDTVdQ	GYQCfSETSH LWGQYAPFFS LANESvISPD	VPaGCRVTFa
<i>A. fumigatus</i> ATCC13073	GskSCDTVd1	GYQCsPATSH LWGQYSPFFS LEDELSVSSK	LPMDCRITLV
<i>A. fumigatus</i> ATCC32722	GskSCDTVd1	GYQCsPATSH LWGQYSPFFS LEDELSVSSK	LPMDCRITLV
<i>A. fumigatus</i> ATCC58128	GskSCDTVd1	GYQCsPATSH LWGQYSPFFS LEDELSVSSK	LPMDCRITLV
<i>A. fumigatus</i> ATCC26906	GskSCDTVd1	GYQCsPATSH LWGQYSPFFS LEDELSVSSK	LPMDCRITLV
<i>A. fumigatus</i> ATCC32239	GskACDTVd1	GYQCsPGtSH LWGQYSPFFS LEDELSVSSD	LPMDCRVTFV
<i>E. nidulans</i>	QNHSCNTAdg	GYQCfPNVSH VWGQYSPYFS IEQESAISd	VPhGCeVTFV
<i>T. thermophilus</i>	DSHSCNTVEg	GYQCrPEISH sWGQYSPFFS LADQSEISPD	VPQCKITFV
<i>T. lanuginosa</i>	-----	-----nvDIAR hWGQYSPFFS LAEvSEISPA	VPKGCrvEFV
<i>M. thermophila</i>	ESRPCDTpD1	GFQCgTAISH FWGQYSPYFS VPSElDaS..	IPDDCeVTFa

Consensus Seq. 11 NSHSCDTVd- GYQC-PEISH LWGQYSPFFS LADESAISPD VPKGCrvTFV

	51		100
<i>P. involutus</i> (phyA1)	NIIqRHGARF	PTSGaTtRik AgLtKLQgvq nftDAKFNF1	KSFKYdLGns
<i>P. involutus</i> (phyA2)	NIIqRHGARF	PTSGaAtRik AgLsKLQsvq nftDPKFDFI	KSFTYdLGTs
<i>T. pubescens</i>	HIIqRHGARF	PTSGaAKRiq TaVAKLKaaS nytdPLlAFV	tnYtYSLGqD
<i>A. pediades</i>	NIIqRHGARF	PTSGaGtRiq AaVKKLQsak TytdPRLDfL	tnYtYTLGhD
<i>P. lycii</i>	NLIqRHGARW	PTSGarsRqv AaVAKIQmar PftDPKYEFL	NdFvYkFGvA
<i>A. terreus</i> 9a1	QVLARHGARS	PThSKTKaYA AtIAaIQKSA TaFpGKYAFL	QSNYSLDSE
<i>A. terreus</i> cbs	QVLARHGARS	PTdSKTKaYA AtIAaIQKNA TaLpGKYAFL	KSNNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTeSKGKKYS ALIEeIQONv TtFDGKYAFL	KTNNYSLGAD
<i>A. niger</i> T213	QVLSRHGARY	PTeSKGKKYS ALIEeIQONv TtFDGKYAFL	KTNNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTdSKGKKYS ALIEeIQONv TtFDGKYAFL	KTNNYSLGAD
<i>A. fumigatus</i> ATCC13073	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTNNYTLGAD
<i>A. fumigatus</i> ATCC32722	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTNNYTLGAD
<i>A. fumigatus</i> ATCC58128	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTNNYTLGAD
<i>A. fumigatus</i> ATCC26906	QVLSRHGARY	PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTNNYTLGAD
<i>A. fumigatus</i> ATCC32239	QVLSRHGARY	PTASKSKKYk kLVtaIQKNA TeFKGKFAFL	ETNNYTLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTeSKSKaYS GLIEaIQKNA TsFwGQYAF	ESNNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKTELYS qLIsRIQKtA TaYKGyYAF	KdYrYqLGAN
<i>T. lanuginosa</i>	QVLSRHGARY	PTAhKSEvYA ELLQRIQDta TeFKGDFaFL	RdYayhLGAD
<i>M. thermophila</i>	QVLSRHGARA	PTlkRAasYv DLIDRIHhGA isYgPgYEFL	RTYDYTLGAD

Consensus Seq. 11 QVLSRHGARY PTSSKSKKYs ALIERIQKNA T-FKGKYAFL KTYNYTLGAD

101

150		
<i>P. involutus</i> (phyA1)	DLvPFGAaQs fDAGQeFaR YskLvSKNnL PFIRadGSDR	VVDSAtNWtA
<i>P. involutus</i> (phyA2)	DLvPFGAaQs fDAGLevFaR YskLvSsDnL PFIRsdGSDR	VVDTAtNWtA
<i>T. pubescens</i>	sLveLGAtQs sEAGQeAftR YsSLvSaDeL PFVRASGSDR	VVATANNWtA
<i>A. pediades</i>	DLvPFGAlQs sQAGeEtFQR YsfLvSKEnL PFVRASSSNR	VVDSAtNWtE
<i>P. lycii</i>	DLlPFGANQs hQTGTDMYtr YsTLfEgGdV PFVRAAGdQR	VVDSStNWtA
<i>A. terreus</i> 9a1	ELTPFGGrNQL rDlGaQFYeR YNAL.TRHIn PFVRATDAsR	VhESAeKFVE
<i>A. terreus</i> cbs	NLTpFGGrNQL qDlGaQFYRR YDTL.TRHIn PFVRAADSSr	VhESAeKFVE
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL VNSGIKFYQR YESL.TRNII	PFIRSSGSsr VIASGEKFIE
<i>A. niger</i> T213	DLTPFGEQEL VNSGIKFYQR YESL.TRNII	PFIRSSGSsr VIASGEKFIE
<i>A. niger</i> NRRL3135	DLTPFGEQEL VNSGIKFYQR YESL.TRNIV	PFIRSSGSsr VIASGKKFIE
<i>A. fumigatus</i> ATCC13073	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR VIASGEKFIE
<i>A. fumigatus</i> ATCC32722	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR VIASGEKFIE
<i>A. fumigatus</i> ATCC58128	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR VIASGEKFIE

Fig. 8A

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A. fumigatus ATCC26906 DLTAFGEQQL VNSGIKFYQR YKAL.ARSVV PFIRASGSDR VIASGEKFIE
 A. fumigatus ATCC32239 DLTTFGEQOM VNSGIKFYQK YKAL.AgSVV PFIRSSGSDR VIASGEKFIE
 E. nidulans DLTIFGENQM VDSGaKPYRR YKAL.ARKnt PFIRASGSDR VVASAEKFIN
 T. thermophilus DLTPFGENQM IOLGIKFYNH YKSL.ARNv PFVRCGSDR VIASGrLFIE
 T. lanuginosa NLTRFGEEQM MESGrQFYHR YREQ.AREIV PFVRAAGSAR VIASAEfFnr
 M. thermophila ELTRtGQQOM VNSGIKFYRR YRAL.ARKsI PFVRTAGqDR VVHSAENftQ

Consensus Seq. 11

DLTPFGENQM VNSGIKFYRR YKAL-ARNIV PFVRASGSDR VIASAEKFIE

P. involutus (phyA1)
 P. involutus (phyA2)
 T. pubescens
 A. pediades
 P. lycii
 A. terreus 9a1
 A. terreus cbs
 A. niger var. awamori
 A. niger T213
 A. niger NRRL3135
 A. fumigatus ATCC13073
 A. fumigatus ATCC32722
 A. fumigatus ATCC58128
 A. fumigatus ATCC26906
 A. fumigatus ATCC32239
 E. nidulans
 T. thermophilus
 T. lanuginosa
 M. thermophila

151
 GFaSA..... .shNtvqPk LNLILPQ..T gNDTLEDNMC PAaGD..... 200
 GFaSA..... .srNaiqPk LDLILPQ..T gNDTLEDNMC PAaGE.....
 GFaLA..... .ssNsitPV LSVIIE..A gNDTLDDNMC PAaGD.....
 GFsAA..... .shHvlnPI LfVILSE..S LNDTLDDAMC PnaGs.....
 GFgdA..... .sgEtvlpT LQVVLQE..E gNcTLcNNMC PnevD.....
 GFQTARqDDh hAnpHQPSPr VDVAIPEGSA YNNTLEHSLC TAFES...ST
 GFQNArqGDP hAnpHQPSPr VDVVIEGTA YNNTLEHSLC TAFE...ST
 GFQSTKLkDP rAqpgQSSPk IDVVIIEASS sNNTLDpGtC TvFED...Se
 GFQSTKLkDP rAqpgQSSPk IDVVIIEASS sNNTLDpGtC TvFED...Se
 GFQSTKLkDP rAqpgQSSPk IDVVIIEASS sNNTLDpGtC TvFED...Se
 GFQqAKLADP gAt.NRAAPa ISVVIIESeT FNNTLDHGVC TkFEA...Sq
 GFQqAKLADP gAt.NRAAPa ISVVIIESeT FNNTLDHGVC TkFEA...Sq
 GFQqAKLADP gAt.NRAAPa ISVVIIESeT FNNTLDHGVC TkFEA...Sq
 GFQqAKLADP gAt.NRAAPa ISVVIIESeT FNNTLDHGVC TkFEA...Sq
 GFQqANVADP gAt.NRAAPV ISVVIIESeT YNNTLDHVC TnFEA...Se
 GFRkaQLhDh g.s.gQATPV VNVIIPEidG FNNTLDHStC vSFEN...de
 GFQSAKVlDP hSdKHDAPpt INVIIIEEGPS YNNTLDtGSc PvFED...SS
 GFQdAKdrDP rSnkDQAEpV INVIIIEETG sNNTLDgltC PAaEE...AP
 GFHSALLADR gStvRPTlPy dmVVIPETAG aNNTLHNDLC TAFEegpyST

Consensus Seq. 11

GFQSAKLADP -A--HQASPV INVIIIEGSG YNNTLDHGLC TAFED---ST

P. involutus (phyA1)
 P. involutus (phyA2)
 T. pubescens
 A. pediades
 P. lycii
 A. terreus 9a1
 A. terreus cbs
 A. niger var. awamori
 A. niger T213
 A. niger NRRL3135
 A. fumigatus ATCC13073
 A. fumigatus ATCC32722
 A. fumigatus ATCC58128
 A. fumigatus ATCC26906
 A. fumigatus ATCC32239
 E. nidulans
 T. thermophilus
 T. lanuginosa
 M. thermophila

201
 .SDpqvnaWl AVafPSItAR LNAAaPSVNL TDtDafNLVs LCAFlTVSK.
 .SDpqvDaWl AsafPSVtAQ LNAAaPGaNL TDADafNLVs LCPFmTVSK.
 .SDpqvQWl AqFAPPMtAR LNAGaPGaNL TDtDtyNLLT LCPFETVat.
 .SDpqtGiWT SIYGTPIanR LNqqaPGaNI TAADVsnLip LCAFETivK.
 .GDESt.twl GVFAPhItAR LNAAaPSaNL SDsDaLtLMD MCPFDTLSS.
 VGDDAVANFT AVFAPAIaQR LEAdLPGVQL LEAdLPGVQL SADDVVNLMA MCPFETVSLT
 VGDAADNFT AVFAPAIaQR LEAdLPGVQL LEAdLPGVQL SADDVVNLMA MCPFETVSLT
 LADtVEANFT AtFAPSIRqR LEndLSGvtL TDtEVtyLMD MCSFDTISTs
 LADtVEANFT AtFAPSIRqR LEndLSGvtL TDtEVtyLMD MCSFDTISTs
 LADtVEANFT AtFAPSIRqR LEndLSGvtL TDtEVtyLMD MCSFDTISTs
 LGDEVAANFT ALFAPDIRAR aEkhlPGvtL TDEDVVS LMD MCSFDTVART
 LGDEVAANFT ALFAPDIRAR aEkhlPGvtL TDEDVVS LMD MCSFDTVART
 LGDEVAANFT ALFAPDIRAR aEkhlPGvtL TDEDVVS LMD MCSFDTVART
 LGDEVAANFT ALFAPDIRAR aKkhlPGvtL TDEDVVS LMD MCSFDTVART
 LGDEVAANFT ALFAPDIRAR IEkhlPGVQL TDDDVVS LMD MCSFDTVART
 rADEiEANFT AIMGPPiRKR LEndLPGIKL TNENViY LMD MCSFDTMART
 gGHDAQEKFA kqFAPAILEK IKDhLPGVDL AvsDVpyLMD LCPFETLARN
 .DptqpAEFl qVFGPRVlKk ItkhMPGVNL TLEDVplFMD LCPFDTVGsd
 IGDDAQDtyl StFAGPItAR VNANLPGaNL TDADtValMD LCPFETVAss

Consensus Seq. 11

LGDDAEANFT AVFAPPiRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVART

P. involutus (phyA1)
 P. involutus (phyA2)
 T. pubescens
 A. pediades
 P. lycii
 A. terreus 9a1
 A. terreus cbs
 A. niger var. awamori

251
ekkSdF CtLFegIPGs FeaFAYggdL dKFYgtGyGQ
eqkSdF CtLFegIPGs FeaFAYagdL dKFYgtGyGQ
errSeF CDIYeelqAE .daFAYnadL dKFYgtGyGQ
etpSPF CNLF..TPEE FaQFEYFgdL dKFYgtGyGQ
gnaSPF CDLF..TAEZ YvsYEXYdL dKYYgtGPGN
 dD..Aht... .LSPF CDLF..TAEZ WtQYNYLlSL dKYYGYGGGN
 dD..Aht... .LSPF CDLF..TAAZ WtQYNYLlSL dKYYGYGGGN
 Tv..DTK... .LSPF CDLF..ThDE WiHYDYLOSL kKYYGHGAGN

Fig. 8B

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A. niger T213	Tv..DTK...LSPF	CDLF..ThDE	WiHYDYLRSL	kKYYGHGAGN
A. niger NRRL3135	Tv..DTK...LSPF	CDLF..ThDE	WiNYDYLRSL	kKYYGHGAGN
A. fumigatus ATCC13073	SD..ASQ...LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC32722	SD..ASQ...LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC58128	SD..ASQ...LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC26906	SD..ASQ...LSPF	CQLF..ThNE	WkKYNYLQSL	gKYYGYGAGN
A. fumigatus ATCC32239	AD..ASE...LSPF	CAIF..ThNE	WkKYDYLRSL	gKYYGYGAGN
E. nidulans	AH..GTE...LSPF	CAIF..TEKE	WlQYDYLRSL	sKYYGYGAGS
T. thermophilus	ht..DT....LSPF	CALs..TqEE	WqAYDYQSL	gKYYGnGGGN
T. lanuginosa	PvlfPrQ...LSPF	CHLF..TADD	WmaYDYyTL	dkYYSHGGGS
M. thermophila	SsdpaTadag	ggngxrplSPF	CrLF..Sese	WraYDYLRSL	gKYYGYGPGN

Consensus Seq. 11 SD--ATQ--- -----LSPF CDLF--TADE W-QYDYLRSL -KYYGYGAGN

	301			350
P. involutus (phyA1)	eLGPvQGVGY	vNELIARLTN	S.AVRDNTqT	NRTLDASpVT FPLNkTfYAD
P. involutus (phyA2)	ALGPvQGVGY	iNELIARLTN	S.AVNDNTqT	NRTLDAApDT FPLNkTMYAD
T. pubescens	PLGPvQGVGY	iNELIARLTa	q.nVsDHTqT	NsTLDSSPET FPLNrTLYAD
A. pediades	PLGPvQGVGY	iNELIARLTg	m.PVRDNTqT	NRTLDSSpIT FPLDrSIYAD
P. lycii	ALGPvQGVGY	vNELIARLTg	q.AVRDETqT	NRTLDSDPAT FPLNrTfYAD
A. terreus 9a1	PLGPvQGVGW	aNELMARLTR	A.PVHDHTCv	NNTLDASPAT FPLNATLYAD
A. terreus cbs	PLGPvQGVGW	aNELIARLTR	S.PVHDHTCv	NNTLDANPAT FPLNATLYAD
A. niger var. awamori	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSNPAT FPLNSTLYAD
A. niger T213	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSNPAT FPLNSTLYAD
A. niger NRRL3135	PLGPTQGVGY	aNELIARLTH	S.PVHDDTSS	NHTLDSSPAT FPLNSTLYAD
A. fumigatus ATCC13073	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMyvD
A. fumigatus ATCC32722	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMyvD
A. fumigatus ATCC58128	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMyvD
A. fumigatus ATCC26906	PLGPAQGIGF	tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMyvD
A. fumigatus ATCC32239	PLGPAQGIGF	tNELIARLTN	S.PVQDHTST	NsTLDSNPAT FPLNATMyvD
E. nidulans	PLGPAQGIGF	tNELIARLTQ	S.PVQDHTST	NHTLDSNPAT FPLDrkLYAD
T. thermophilus	PLGPAQGVGF	vNELIARMTg	S.PVQDYTTv	NHTLDSNPAT FPLNATLYAD
T. lanuginosa	AFGPSRGVGF	vNELIARMTg	NlPVKDHTT	NHTLDdNPET FPLDAvLYAD
M. thermophila	PLGPTQGVGF	vNELIARLA	GvPVRDgTST	NRTLDGDPtT FPLGrPLYAD

Consensus Seq. 11 PLGPAQGVGF -NELIARLTH S-PVQDHTST NHTLDSNPAT FPLNATLYAD

	351			400
P. involutus (phyA1)	FSHDNlMVAV	FsAMGLFrqP	aPLSTsvNP	wrt.....Wr TSSlVPFSGR
P. involutus (phyA2)	FSHDNlMVAV	FsAMGLFrqS	aPLSTSTpDP	nrt.....Wl TSSvVPFSAR
T. pubescens	FSHDNqMVAI	FsAMGLFNqS	aPLdPTTpDP	art.....Fl vkkiVPFSAR
A. pediades	LSHDNqMIAI	FsAMGLFNqS	sPLdPSfpNP	krt.....Wv TSRLtPFSAR
P. lycii	FSHDNTMVPI	FaALGLFNAT	a.LdPlkpDe	nrl.....Wv DSKlVPFSGH
A. terreus 9a1	FSHDSnLVSI	FWALGLYNGT	aPLSqtSVES	Vs..QTDGYA AAWTVPFAAR
A. terreus cbs	FSHDSnLVSI	FWALGLYNGT	KPLSqtTVed	It..rTDGYA AAWTVPFAAR
A. niger var. awamori	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
A. niger T213	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
A. niger NRRL3135	FSHDNGIISI	LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
A. fumigatus ATCC13073	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ELDGYS ASWvVPFGAR
A. fumigatus ATCC32722	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ELDGYS ASWvVPFGAR
A. fumigatus ATCC58128	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ELDGYS ASWvVPFGAR
A. fumigatus ATCC26906	FSHDNSMVSI	FFALGLYNGT	EPLSrTSVES	ak..ELDGYS ASWvVPFGAR
A. fumigatus ATCC32239	FSHDNGMIPI	FFAMGLYNGT	EPLSqtSeES	tk..ESNGYS ASWAVPFGAR
E. nidulans	FSHDNSMISI	FFAMGLYNGT	QPLSmdSVES	Iq..EmDGYA ASWTVPFGAR
T. thermophilus	FSHDNTMtSI	FaALGLYNGT	akLSTTeIKS	Ie..ETDGYA AAWTVPFGGR
T. lanuginosa	FSHDNTMtGI	FsAMGLYNGT	KPLSTSKIQP	ptgaAADGYA ASWTVPFAAR
M. thermophila	FSHDNnMMGV	LgALGayDgV	pPLdkTArrd	..peELGGYA ASWAVPFAAR

Consensus Seq. 11 FSHDNTMVSI FFALGLYNGT KPLSTTSVES I---ETDGYA ASWTVPFAAR

	401			450
P. involutus (phyA1)	mvVERLsC..	fGt.....	Tk	VRVLVQDQVq PLEfCGgDRn
P. involutus (phyA2)	maVERLsC..	AGt.....	Tk	VRVLVQDQVq PLEfCGgDQd
T. pubescens	mvVERLDC..	GGa.....	Qs	VRLLVNDaVq PLafCGaDts

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<i>A. pediades</i>	mvtErLlCQr	DGtGsGGpsr	imrNgnvQTF	VRILVNDaLq	PLkfCGgDmd
<i>P. lycii</i>	mtVEkLaC...sgKea	VRVLVNDaVq	PLEfCGg.vd	
<i>A. terreus</i> 9a1	AYVEMMQCrAEK...	EPL VRVLVNDVRM	PLHGCPtDKL	
<i>A. terreus</i> cbs	AYIEMMQCrAEK...	QPL VRVLVNDVRM	PLHGCAVDNL	
<i>A. niger</i> var. <i>awamori</i>	1YVEMMQCQAEQ...	EPL VRVLVNDRVV	PLHGCPIDaL	
<i>A. niger</i> T213	1YVEMMQCQAEQ...	EPL VRVLVNDRVV	PLHGCPIDaL	
<i>A. niger</i> NRRL3135	1YVEMMQCQAEQ...	EPL VRVLVNDRVV	PLHGCPVdaL	
<i>A. fumigatus</i> ATCC13073	AYfEtMQCKSEK...	EPL VRaLINDRVV	PLHGCDVDKL	
<i>A. fumigatus</i> ATCC32722	AYfEtMQCKSEK...	EPL VRaLINDRVV	PLHGCDVDKL	
<i>A. fumigatus</i> ATCC58128	AYfEtMQCKSEK...	ESL VRaLINDRVV	PLHGCDVDKL	
<i>A. fumigatus</i> ATCC26906	AYfEtMQCKSEK...	EPL VRaLINDRVV	PLHGCDVDKL	
<i>A. fumigatus</i> ATCC32239	AYfEtMQCKSEK...	EPL VRaLINDRVV	PLHGCAVDKL	
<i>E. nidulans</i>	AYfELMQCE.KK...	EPL VRVLVNDRVV	PLHGCAVDKF	
<i>T. thermophilus</i>	AYIEMMQCDDsD...	EPV VRVLVNDRVV	PLHGCEVDsL	
<i>T. lanuginosa</i>	AYVELLRcET	ETsSeEEeEG	..ED...	EPF VRVLVNDRVV	PLHGCrVDRW
<i>M. thermophila</i>	iYVEkMRCsG	GGgGGGGgEG	..rQekDeem	VRVLVNDVRM	TLkGCGaDer

Consensus Seq. 11

AYVEMMQCEA GG-G-GG-EG --EK---EPL VRVLVNDRVV PLHGCGVDKL

	451	482	
<i>P. involutus</i> (phyA1)	GlCtLAKFVE	SqTFARSDga	GDFEKCFAts a-
<i>P. involutus</i> (phyA2)	GlCaLDKFVE	SqAYARSGga	GDFEKCLAtt v-
<i>T. pubescens</i>	GvCtLDAFVE	SqAYARNDge	GDFEKCFAt- --
<i>A. pediades</i>	SlCtLEAFVE	SqkYAReDgq	GDFEKCFD-- --
<i>P. lycii</i>	GvCELSAFVE	SqTYAReNgq	GDFAKCgfv se
<i>A. terreus</i> 9a1	GRCKrDAFVA	GLSFAQAG..	GNWADCF--- --
<i>A. terreus</i> cbs	GRCKrDDFVE	GLSFARAG..	GNWAECE--- --
<i>A. niger</i> var. <i>awamori</i>	GRCtrDsFVr	GLSFARSG..	GDWAECSA--- --
<i>A. niger</i> T213	GRCtrDsFVr	GLSFARSG..	GDWAECEFA--- --
<i>A. niger</i> NRRL3135	GRCtrDsFVr	GLSFARSG..	GDWAECEFA--- --
<i>A. fumigatus</i> ATCC13073	GRCKLNDFVK	GLSWARSG..	GNWGECFS--- --
<i>A. fumigatus</i> ATCC32722	GRCKLNDFVK	GLSWARSG..	GNWGECFS--- --
<i>A. fumigatus</i> ATCC58128	GRCKLNDFVK	GLSWARSG..	GNWGECFS--- --
<i>A. fumigatus</i> ATCC26906	GRCKLNDFVK	GLSWARSG..	GNWGECFS--- --
<i>A. fumigatus</i> ATCC32239	GRCKLKDFVK	GLSWARSG..	GNSEQSFS--- --
<i>E. nidulans</i>	GRCTLDDWVE	GLNFARSG..	GNWktCFTl- --
<i>T. thermophilus</i>	GRCKrDDFVr	GLSFARqG..	GNWEGCYAas e-
<i>T. lanuginosa</i>	GRCRrDEWIK	GLTFARqG..	GHWDrcF--- --
<i>M. thermophila</i>	GmCtLErFIE	SMAFARGN..	GKWDlCFA--- --

Consensus Seq. 11

GRCKLDDFVE GLSFARSG-- GNWAECEFA--- --

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M G V F V V L L S I A T L F G S T S G T 20
ATGGGCGTGTTCGTCTGCTACTGTCCATTGCCACCTTGTTCGGTTCACATCCGGTACC
1 ---+-----+-----+-----+-----+-----+----- 60
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGAACAAGCCAAGGTGTAGGCCATGG

A L G P R G N S H S C D T V D G G Y Q C 40
GCCTTGGGTCCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61 ---+-----+-----+-----+-----+-----+----- 120
CGGAACCCAGGAGCACCATTAAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

F P E I S H L W G T Y S P Y F S L A D E 60
TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA
121 ---+-----+-----+-----+-----+-----+----- 180
AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

S A I S P D V P D D C R V T F V Q V L S 80
TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTTCGTTCAAGTTTGTCT
187 ---+-----+-----+-----+-----+-----+----- 240
AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

R H G A R Y P T S S A S K A Y S A L I E 100
AGACACGGTGCTAGATACCCAATTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
241 ---+-----+-----+-----+-----+-----+----- 300
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAACTAATT

A I Q K N A T A F K G K Y A F L K T Y N 120
GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
301 ---+-----+-----+-----+-----+-----+----- 360
CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAATTCTGAATGTTG

Y T L G A D D L T P F G E N Q M V N S G 140
TACACTTTGGGTGCTGACGACTTGACTCCATTGCGGTGAAAACCAATGGTTAACTCTGGT
361 ---+-----+-----+-----+-----+-----+----- 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

I K F Y R R Y K A L A R K I V P F I R A 160
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCAATTAGAGCT
421 ---+-----+-----+-----+-----+-----+----- 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

S G S D R V I A S A E K F I E G F Q S A 180
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTTCAAGGTTTCCAATCTGCT
481 ---+-----+-----+-----+-----+-----+----- 540
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAGGTTAGACGA

K L A D P G S Q P H Q A S P V I N V I I 200
AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT
541 ---+-----+-----+-----+-----+-----+----- 600
TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA

P E G S G Y N N T L D H G T C T A F E D 220
CCAGAAGGATCCGTTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTTGAAGAC
601 ---+-----+-----+-----+-----+-----+----- 660
GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG

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S E L G D D V E A N F T A L F A P A I R 240
TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTCCAGCTATTAGA
661 ---+-----+-----+-----+-----+-----+-----+----- 720
AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTGCGATAATCT

A R L E A D L P G V T L T D E D V V Y L 260
GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG
721 ---+-----+-----+-----+-----+-----+-----+----- 780
CGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGCTTCTGCAACAAATGAAC

M D M C P F D T V A R T S D A T E L S P 280
ATGGACATGTGTCCATTGACACTGTGCTAGAACTTCTGACGCTACTGAATTGTCTCCA
781 ---+-----+-----+-----+-----+-----+-----+----- 840
TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

F C A L F T H D E W I Q Y D Y L Q S L G 300
TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT
841 ---+-----+-----+-----+-----+-----+-----+----- 900
AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

K Y Y G Y G A G N P L G P A Q G V G F A 320
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGTTTCGCT
901 ---+-----+-----+-----+-----+-----+-----+----- 960
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCACACAACCAAGCGA

N E L I A R L T H S P V Q D H T S T N H 340
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC
961 ---+-----+-----+-----+-----+-----+-----+----- 1020
TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

T L D S N P A T F P L N A T L Y A D F S 360
ACTTTGGACTCTAACCCAGCTACTTTCCATTGAACGCTACTTTGTACGCTGACTTCTCT
1021 ---+-----+-----+-----+-----+-----+-----+----- 1080
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACCTTGCGATGAAACATGCGACTGAAGAGA

H D N T M I S I F F A L G L Y N G T K P 380
CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA
1081 ---+-----+-----+-----+-----+-----+-----+----- 1140
GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT

L S T T S V E S I E E T D G Y S A S W T 400
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT
1141 ---+-----+-----+-----+-----+-----+-----+----- 1200
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

V P F A A R A Y V E M M Q C Q A E K E P 420
GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA
1201 ---+-----+-----+-----+-----+-----+-----+----- 1260
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTTCGACTTTTCCTTGGT

L V R V L V N D R V V P L H G C A V D K 440
TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG
1261 ---+-----+-----+-----+-----+-----+-----+----- 1320
AACCAATCTCAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTC

L G R C K R D D F V E G L S F A R S G G 460

21/32

```
TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTAGATCTGGTGGT
1321 ----+-----+-----+-----+-----+-----+-----+----- 1380
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

N W A E C F A * 467
AACTGGGCTGAATGTTTCGCTTAA
1381 ----+-----+-----+-----+ 1410
TTGACCCGACTTACAAAGCGAATT
```

22/32

M G V F V V L L S I A T L F G S T S G T 20
ATGGGCGTGTTCGTGCTGCTACTGTCCATTGCCACCTTGTTCCGGTTCACATCCGGTACC 60
1 -----+-----+-----+-----+-----+-----+-----+
TACCCGCACAAGCAGCAGCATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG
A L G P R G N S H S C D T V D G G Y Q C 40
GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT -
61 -----+-----+-----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTGAGAGTGAGAACAACACTGTGACAACCTGCCACCAATGGTTACA
A F P E I S H L W G T Y S P F F S L A D E 60
TTCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTGGCTGACGAA
121 -----+-----+-----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT
S A I S P D V P K G C R V T F V Q V L S 80
TCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTTACTTTTCGTTCAAGTTTTGTCT
181 -----+-----+-----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA
R H G A R Y P T S S A S K A Y S A L I E 100
AGACACGGTGTCTAGATACCCAACCTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGCATGAGACGAAACTAACTT
A I Q K N A T A F K G K Y A F L K T Y N 120
GCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
301 -----+-----+-----+-----+-----+-----+-----+ 360
CGATAAGTTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG
A Y T L G A D D L T P F G E Q Q M V N S G 140
TACACTTTGGGTGCTGACGACTTGACTCCATTCCGGTGAACAACAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTGTTTACCAATTGAGACCA
I K F Y R R Y K A L A R K I V P F I R A 160
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCAATAGAGCT
421 -----+-----+-----+-----+-----+-----+-----+ 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA
S G S D R V I A S A E K F I E G F Q S A 180
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTTCAATGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAGGTTAGACGA
K L A D P G A N P H Q A S P V I N V I I 200
AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT
541 -----+-----+-----+-----+-----+-----+-----+ 600
TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA
P E G A G Y N N T L D H G L C T A F E E 220
CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTTGAAGAA
601 -----+-----+-----+-----+-----+-----+-----+ 660
GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT

23/32

S E L G D D V E A N F T A V F A P P I R 240
TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTTCGCTCCACCAATTAGA
661 -----+-----+-----+-----+-----+-----+-----+ 720
AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

A R L E A H L P G V N L T D E D V V N L 260
GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTTAACTTG
721 -----+-----+-----+-----+-----+-----+-----+ 780
CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

M D M C P F D T V A R T S D A T Q L S P 280
ATGGACATGTGTCCATTTCGACACTGTTGCTAGAAGTCTGACGCTACTCAATTGTCTCCA
781 -----+-----+-----+-----+-----+-----+-----+ 840
TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

F C D L F T H D E W I Q Y D Y L Q S L G 300
TTCTGTGACTTGTTCACCTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT
841 -----+-----+-----+-----+-----+-----+-----+ 900
AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

K Y Y G Y G A G N P L G P A Q G V G F V 320
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT
901 -----+-----+-----+-----+-----+-----+-----+ 960
TTCATGATGCCAATGCCACGACCATTGGGTAAACCCAGGTTCGAGTTCCACAACCAAAGCAA

N E L I A R L T H S P V Q D H T S T N H 340
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAAACAC
961 -----+-----+-----+-----+-----+-----+-----+ 1020
TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

T L D S N P A T F P L N A T L Y A D F S 360
ACTTTGGACTCTAACCAGCTACTTTCCCATGAAACGCTACTTTGTACGCTGACTTCTCT
1021 -----+-----+-----+-----+-----+-----+-----+ 1080
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAAGTTGCGATGAAACATGCGACTGAAGAGA

H D N T M V S I F F A L G L Y N G T K P 380
CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA
1081 -----+-----+-----+-----+-----+-----+-----+ 1140
GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCCGT

L S T T S V E S I E E T D G Y S A S W T 400
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT
1141 -----+-----+-----+-----+-----+-----+-----+ 1200
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

V P F A A R A Y V E M M Q C E A E K E P 420
GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA
1201 -----+-----+-----+-----+-----+-----+-----+ 1260
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

L V R V L V N D R V V P L H G C G V D K 440
TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTGACAAG
1261 -----+-----+-----+-----+-----+-----+-----+ 1320
AACCAATCTCAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACCTGTTT

L G R C K R D D F V E G L S F A R S G G 460

24/32

TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 -----+-----+-----+-----+-----+-----+ 1380
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

N W E E C F A * 467
AACTGGGAAGAATGTTTCGCTTAA
1381 -----+-----+----- 1404
TTGACCCTTCTTACAAAGCGAATT

25/32

M G V F V V L L S I A T L F G S T S G T 20
ATGGGGGGTTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTCGGCAGCACATCGGGCACT
1 -----+-----+-----+-----+-----+ 60
TACCCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA

A L G P R G N H S K S C D T V D L G Y Q 40
GCGCTGGGCCCCCGTGGAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG -
61 -----+-----+-----+-----+-----+ 120
CGCGACCCGGGGGCACCTTTAGTGAGGTTCCAGGACGCTATGCCATCTGGATCCCATGGTC

C S P A T S H L W G T Y S P Y F S L E D 60
TGCTCCCCTGCGACTTCTCATCTATGGGGCAGTACTCGCCATaCTTTTCGCTCGAGGAC
121 -----+-----+-----+-----+-----+ 180
ACGAGGGGACGCTGAAGAGTAGATACCCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG

E L S V S S K L P K D C R I T L V Q V L 80
GAGCTGTCCGTGTGCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA
181 -----+-----+-----+-----+-----+ 240
CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGAACCATGTCCACGAT

S R H G A R Y P T S S K S K K Y K K L I 100
TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt
241 -----+-----+-----+-----+-----+ 300
AGCGCGGTACCTCGCGCCATGGGTTGGTTCGAGGTTCTCGTTTTTCATATTCTTGAAtAa

T A I Q A N A T D F K G K Y A F L K T Y 120
ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTAcGCCTTTTTGAAGACGTAC
301 -----+-----+-----+-----+-----+ 360
TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCatgCGGAAAACTTCTGCATG

N Y T L G A D D L T P F G E Q Q L V N S 140
AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACCTCG
361 -----+-----+-----+-----+-----+ 420
TTGATATGAGACCCACGCCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC

G I K F Y Q R Y K A L A R S V V P F I R 160
GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTTCGC
421 -----+-----+-----+-----+-----+ 480
CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG

A S G S D R V I A S G E K F I E G F Q Q 180
GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTCATCGAGGGGTTCAGCAG
481 -----+-----+-----+-----+-----+ 540
CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTGCTC

A K L A D P G A T N R A A P A I S V I I 200
GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCGCTCCGGCGATTAGTGTGATTATT
541 -----+-----+-----+-----+-----+ 600
CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA

P E S E T F N N T L D H G V C T K F E A 220
CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG
601 -----+-----+-----+-----+-----+ 660
GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACCTCCCG

26/32

S Q L G D E V A A N F T A L F A P D I R 240
ATGCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA
661 -----+-----+-----+-----+-----+-----+ 720
TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

A R L E K H L P G V T L T D E D V V S L 260
GCTCGCctCGAGAAGCATCTTCTGCGGTGACGCTGACAGACGAGGACGTTGTCACTCTA
721 -----+-----+-----+-----+-----+-----+ 780
CGAGCGgaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

M D M C P F D T V A R T S D A S Q L S P 280
ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCAACG
781 -----+-----+-----+-----+-----+-----+ 840
TACCTGTACACagGCAAACTATGCCATCGCGCGTGGTTCGCTGCGTTCACTCGACAGTGGC

F C Q L F T H N E W K K Y D Y L Q S L G 300
TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC
841 -----+-----+-----+-----+-----+-----+ 900
AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

K Y Y G Y G A G N P L G P A Q G I G F T 320
AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCAAC
901 -----+-----+-----+-----+-----+-----+ 960
TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCCTATCCCAAGTGG

N E L I A R L T R S P V Q D H T S T N S 340
AACGAGCTGATTGCCCCGTTGACgCGTTCGCCAGTGACAGGACCACACCAGCACTAACTCG
961 -----+-----+-----+-----+-----+-----+ 1020
TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTACAGTCCTGGTGTGGTTCGTGATTGAGC

T L V S N P A T F P L N A T M Y V D F S 360
ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTGACTTTTCA
1021 -----+-----+-----+-----+-----+-----+ 1080
TGAGATCAGAGGTTGGGCCGGTGGAAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

H D N S M V S I F F A L G L Y N G T E P 380
CACGACAAACAGCATGGTTTCCATCTTCTTGCATTGGGCCTGTACAACGGCACTGAACCC
1081 -----+-----+-----+-----+-----+-----+ 1140
GTGCTGTTGTCTACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG

L S R T S V E S A K E L D G Y S A S W V 400
TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG
1141 -----+-----+-----+-----+-----+-----+ 1200
AACAGGGCCTGGAGCCACCTTTCGCGGTTCTTAACCTACCCATAAGACGTAGGACCCAC

V P F G A R A Y F E T M Q C K S E K E P 420
GTGCCTTTTCGGCGCGGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT
1201 -----+-----+-----+-----+-----+-----+ 1260
CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTACGCTTTTCTCCTCGGA

L V R A L I N D R V V P L H G C D V D K 440
CTTGTTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG
1261 -----+-----+-----+-----+-----+-----+ 1320
GAACAAGCGCGAACTAATTACTGGCCCCAACACGGTGACGTACCGACGCTACACCTGTTC

L G R C K L N D F V K G L S W A R S G G 460

27/32

CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321 -----+-----+-----+-----+-----+-----+ 1380
GACCCCGCTACGTTGCGACTTACTGAAACAGTCCCTAACTCAACCCGGTCTAGACCCCG

N W G E C F S * 467
AACTGGGGAGAGTGCTTTAGTTGA
1381 -----+-----+----- 1404
TTGACCCCTCTCAGAAATCAACT

28/32

CP-1

Eco RI M G V F V V L L S I A T L F G S T
TATATGAATTCATGGGCGTGTTCGTCGCTACTGTCCATTGCCACCTTGTTCGGTTCCA
-----+-----+-----+-----+-----+-----+ 60
ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGT

S G T A L G P R G N S H S C D T V D G G
CATCCGGTACCGCCTTGGGTCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG
-----+-----+-----+-----+-----+-----+ 120
GTAGGCCATGGCGGAACCCAGGAGCACCATTAAAGAGTGAGAACAACCTGTGACAACCTGCCAC

CP-2

CP-3

Y Q C F P E I S H L W G Q Y S P Y F S L
GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT
-----+-----+-----+-----+-----+-----+ 180
CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCAGTTATGAGAGGTATGAAGAGAA

E D E S A I S P D V P D D C R V T F V Q
TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTT
-----+-----+-----+-----+-----+-----+ 240
ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG

CP-4.7

CP-5.7

V L S R H G A R Y P T D S K G K K Y S A
AAGTTTTGTCTAGACACGGTGTCTAGATACCCAAGTgactCTAAGgggTAAGaagTACTCTG
-----+-----+-----+-----+-----+-----+ 300
TTCAAAACAGATCTGTGCCACGATCTATGGGTTGactgAGATTCCcaTTcttCATGAGAC

L I E A I Q K N A T A F K G K Y A F L K
CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA
-----+-----+-----+-----+-----+-----+ 360
GAAACTAAGTTCGATAAGTTTTCTTGCATGACGAAAGTCCCATTTCATGCGAAAGAAGT

CP-6

CP-7

T Y N Y T L G A D D L T P F G G E N Q M V
AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTGCGGTGAAAACCAAATGG
-----+-----+-----+-----+-----+-----+ 420
TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC

N S G I K F Y R R Y K A L A R K I V P F
TTAAGTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT
-----+-----+-----+-----+-----+-----+ 480
AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA

CP-8.7

CP-9

I R A S G S S R V I A S A E K F I E G F
TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTTCAATTGAAGGTT
-----+-----+-----+-----+-----+-----+ 540
AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA

Q S A K L A D P G S Q P H Q A S P V I D
TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG
-----+-----+-----+-----+-----+-----+ 600
AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTGGAAGAGGTCAATAAC

29/32

CP-10.7CP-11.7

V I I S E A S S Y N N T L D P G T C T A
ACGTTATTATTtctGAcgtTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG
601 -----+-----+-----+-----+-----+ 660
TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC

30/32

F E D S E L A D T V E A N F T A L F A P
CTTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTTCGCTC
661 -----+-----+-----+-----+-----+-----+-----+ 720
GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG
CP-12.7

A I R A R L E A D L P G V T L T D T E V
CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG
721 -----+-----+-----+-----+-----+-----+-----+ 780
GTGCATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGtgacttc

CP-13.7
T Y L M D M C S F E T V A R T S D A T E
TTactTTACTTGATGGACATGTGtctTTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG
781 -----+-----+-----+-----+-----+-----+-----+ 840
AatgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R H Y D Y L Q
AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcactACGACTACTTGC
841 -----+-----+-----+-----+-----+-----+-----+ 900
TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG
CP-14.7

CP-15.7
S L K K Y Y G H G A G N P L G P T Q G V
AATCTTTGaagaAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG
901 -----+-----+-----+-----+-----+-----+-----+ 960
TTAGAAACTtctTTCATGATGCCAgtgCCACGACCATTGGGTAAACCAGGttgaGTTCCAC

G F A N E L I A R L T R S P V Q D H T S
TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT
961 -----+-----+-----+-----+-----+-----+-----+ 1020
AACCAAAGCGATTGCTTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA
CP-16

CP-17.7
T N H T L D S N P A T F P L N A T L Y A
CTACTAACCACTTTGGACTCTAACCCAGCTACTTTCCATTGAACGCTACTTTGTACG
1021 -----+-----+-----+-----+-----+-----+-----+ 1080
GATGATTGGTGTGAAACCTGAGATTGGGTGATGAAAGGGTAACTTGCGATGAAACATGC

D F S H D N G I I S I F F A L G L Y N G
CTGACTTCTCTCACGACAACggtattATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG
1081 -----+-----+-----+-----+-----+-----+-----+ 1140
GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC
CP-18.7

CP-19.7
T A P L S T T S V E S I E E T D G Y S S
GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTt
1141 -----+-----+-----+-----+-----+-----+-----+ 1200
CATGACGAGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGAA

A W T V P F A S R A Y V E M M Q C Q A E
ctgctTGGACTGTTCCATTTCgttctAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG
1201 -----+-----+-----+-----+-----+-----+-----+ 1260
gacgaACCTGACAAGGTAAGcgaagaTCTCGAATGCAACTTTACTACGTTACAGTTTCGAC
CP-20

CP-21

31/32

K E P L V R V L V N D R V V P L H G C A
AAAAGGAACCATTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG
1261 -----+-----+-----+-----+-----+ 1320
TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC

32/32

V D K L G R C K R D D F V E G L S F A R
CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTA
1321 -----+-----+-----+-----+-----+ 1380
GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTCCAAACAGAAAGCGAT
CP-22
S G G N W A E C F A * Eco RI
GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA
1381 -----+-----+-----+-----+----- 1426
CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 99/00154

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23K 1/165, A01H 5/00, C12N 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9716981 A1 (GIST-BROCADES B.V.), 15 May 1997 (15.05.97), See Example 2, page 10; and the claims --	1-4
X	EP 0619369 A1 (AVEVE N.V.), 12 October 1994 (12.10.94), See page 5, lines 11-15; page 7, lines 1-3; and claims 20-21 --	1-14
X	EP 0682876 A1 (SOUFFLET ALLMENTAIRE), 22 November 1995 (22.11.95), See page 3, lines 21-25 and claim 9 --	1-8
X	WO 9114782 A1 (GIST-BROCADES N.V.), 3 October 1991 (03.10.91), See page 2, lines 25-27 --	9-14

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Date of the actual completion of the international search

6 July 1999

Date of mailing of the international search report

13 -07- 1999

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 99/00154

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9735016 A1 (NOVO NORDISK BIOTECH, INC.), 25 Sept 1997 (25.09.97), See page 41, lines 7-8; and claim 40 --	1-14
A	Dialog Information Services, File 5, Biosis, Dialog accession no. 10902627, Biosis accession no. 199799523772, Jiang Junping: "Thermostable phytase from Aspergillus sp.", Weishengwu Xuebao 36 (6): p476-478 1996 -- -----	1-14

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/06/99

International application No.
PCT/DK 99/00154

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EP 0682876 A1	22/11/95	AT 172078 T DE 69505299 D FR 2719978 A,B	15/10/98 00/00/00 24/11/95
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